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INFLUENCES OF ENVIRONMENTAL FACTORS AND REPRODUCTIVE ACTIVITIES ON CIRCULATING LEVELS OF GROWTH HORMONE IN GOLDFISH, *CARASSIUS AURATUS* L.

by

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A THESIS

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ABSTRACT

The present report describes changes in circulating levels of growth hormone (GH) in relation to various environmental factors, and spontaneous ovulation in the goldfish, *Carassius auratus* L. A significant daily cycle in serum GH levels was found only in goldfish sampled in February; at this time of the year, circulating GH concentrations reached peak levels shortly after the onset of the scotophase. Throughout the rest of the year, daily rhythms in serum GH levels were not found.

The mean daily serum GH level in goldfish was found to vary on a seasonal basis. The highest serum GH levels occurred in fish sampled in March and June, whereas the lowest level was found in fish sampled in November. These changes appear to be related to seasonal variations in daylength, and it is suggested that changes in the duration of the photophase or scotophase during the year may be the environmental factor by which seasonal changes in circulating GH levels are regulated.

In goldfish, seasonal variations in growth rates were found that are similar to those reported for other temperate-zone teleost fishes. The growth rates were closely related to changes in water temperature, with the highest growth rates occurring in the summer, and the lowest growth rates occurring in the winter months. There is a lag period of several weeks between seasonal maxima in mean daily serum GH levels and the growth rates, possibly due to the reduced metabolic activity of GH at the lower water temperatures. Female goldfish exhibited faster growth rates than male fish at certain times of the year, but these variations were not related to sexual differences in serum GH levels.

The influence of temperature and photoperiod on body growth and circulating GH levels were studied at various times throughout the year. At all times of the year, increased water temperature usually resulted in an increased growth rate; increased serum GH levels were generally found in fish exposed to the warmer temperature. Photoperiod was shown to modify the growth response to temperature, especially during the early part of the year when goldfish exposed to 20 °C and long photoperiod exhibited higher growth rates than fish exposed to short photoperiod and 20 °C. This suggests that the effect of

photoperiod on the growth rates may vary on a seasonal basis. A consistent effect of photoperiod on serum GH levels similar to that on growth rates was not found.

During spontaneous ovulation in goldfish, the ovulatory surge of gonadotropin (GtH) is accompanied by a substantial increase in the circulating level of GH. In ovulatory goldfish, the plasma GH level increased in a manner similar to the plasma GtH level, but some temporal differences were observed. It is suggested that hypothalamic gonadotropin–releasing hormone (GnRH) caused the release of both GtH and GH from the pituitary, although the mechanism by which GnRH may stimulate GH release is not known.

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I. GENERAL INTRODUCTION

Many teleost fishes found in temperate climatic zones show a marked annual cycle in the growth of somatic and gonadal tissues (Shul'man, 1974; Ricker, 1979; Peter and Crim, 1979). In general, somatic and gonadal growth are alternate activities in spring–spawning teleosts, with gonadal growth occurring in autumn or winter, and somatic growth being fastest in summer and slowest in winter. Metabolic requirements associated with gonadal maturation, seasonal variations in food availability, and seasonal changes in environmental variables such as photoperiod and temperature undoubtedly contribute to the annual pattern of somatic growth in teleosts (Shul'man, 1974; Brett, 1979; Ricker, 1979).

The endocrine control of gonadal growth has received much attention in recent years and the annual cycle of gonadal maturation is known to be regulated by the pituitary hormone gonadotropin (Peter and Crim, 1979; Peter, 1981). Relatively little research, however, has been done on the endocrine regulation of somatic growth in teleost fishes although evidence to date has supported the hypothesis that growth hormone (GH) has an important role in controlling somatic growth (Kayes, 1978; Donaldson *et al.*, 1979; Cook, 1981).

Most of the research concerning GH in teleost fishes has been concerned with the effects of exogenously administered GH (for review: Donaldson *et al.*, 1979), and little work has been done on the influence of endogenous GH on somatic growth. A major factor preventing such work has been the lack of a suitable method for determining circulating levels of GH in fish. Recently, however, a radioimunoassay (RIA) for measuring blood levels of GH in goldfish, *Carassius auratus*, has been developed and validated (Cook *et al.*, 1983), making the measurement of endogenous levels of GH possible in a teleost species.

The present report is the first to attempt to describe changes in circulating levels of GH throughout the annual growth cycle of a teleost fish. The second chapter describes changes in serum levels of GH on a seasonal basis in goldfish and relates these changes to seasonal variations in somatic growth rates. Chapter II also reports changes in serum

concentrations of GH during a complete 24 hour period at various times throughout the year. Chapter III describes the effects of various temperature and photoperiod regimes on the rate of body growth and serum GH levels several times throughout the year in goldfish. Previous reports have also linked GH with reproductive activities of teleost fishes (Chang *et al.*, 1982; Stacey *et al.*, 1983a) and Chapter IV describes variations in blood GH levels associated with spontaneous ovulation in goldfish.

II. DAILY AND SEASONAL VARIATIONS IN CIRCULATING LEVELS OF GROWTH HORMONE IN GOLDFISH, *Carassius auratus*

A. INTRODUCTION

Seasonal growth cycles appear to be universal in teleost species found in temperate climatic zones (Ricker, 1979). Variations in somatic growth rates throughout the year have been characterized for several teleost species, including common carp, *Cyprinus carpio*, (Kawamota *et al.*, 1957), the suckers *Catostomus commersoni* and *C. catostomus* (Basset, 1957), barbel, *Barbus barbus*, (Hunt and Jones, 1975), black crappie, *Pomoxis nigromaculatus*, (Haines, 1980), bluegill sunfish, *Lepomis macrochirus*, (Gerking, 1966), the perch *Perca fluviatilis* and *P. flavescens* (Le Cren, 1951; Kearns and Atchison, 1979), northern pike, *Esox lucius*, (Diana and MacKay, 1978), brown trout, *Salmo trutta*, (Swift, 1961), various coregonid species (Hogman, 1968; Berg, 1970; Hagen, 1970), and several marine teleost species from the Azov and Black Seas (Shul'man, 1974). Despite the diversity in the the species noted above, somatic growth tends to follow the seasons in all species studied, with the highest somatic growth rate occurring in the summer and a very reduced growth rate in the winter.

Food availability, reproductive activities, and environmental variables such as temperature and photoperiod, undoubtedly contribute to the seasonal variations in growth rates (Swift, 1961; Gerking, 1966; Shul'man, 1974; Brett, 1979). Few studies have examined the endocrine regulation of the somatic growth cycle in fishes, although several authors have suggested that seasonal variations in GH may be involved in the growth cycle (Pickford, 1957; Gross *et al.*, 1965; Gerking, 1966; Adelman, 1977; Kayes, 1977; Brett, 1979). A number of studies using indirect measurements of the pituitary content of GH have suggested that the pituitary content of GH may vary on a seasonal basis (Scruggs, 1951; Swift and Pickford, 1965; Bhargava and Raizada, 1973; Kaul and Vollrath, 1974). In these studies, however, corresponding variations in circulating levels of GH could not be determined and the relation of endogenous GH to the growth cycle could not be ascertained.

More recently, Cook (1981) measured serum GH levels in goldfish at three different times in the year and found evidence for a seasonal variation in serum GH levels

in goldfish, with increased serum levels of GH in August and lower levels in February. The experimental design of the study, however, did not allow definitive conclusions to be made regarding the cause of the variations in serum GH levels; in addition, these variations were not correlated with simulataneous changes in somatic growth rates.

Significant daily variations in the circulating levels of a number of hormones in teleost fishes have been described. For example, in goldfish, circulating levels of cortisol (Peter *et al.*, 1978), thyroid hormones (Spieler and Noeske, 1978), gonadotropin (Hontela and Peter, 1978), and prolactin (McKeown and Peter, 1976) have been shown to vary on a daily basis. Few studies have examined similar changes in GH in teleost fishes. Daily variations in plasma GH levels in juvenile kokanee salmon, *Oncorhynchus nerka*, have been described (Leatherland *et al.*, 1974), although the heterologous RIA used in this study is of questionable validity (Nicoll, 1975). In tilapia, *Sarotherodon mossambicus*, diel changes in the nuclear area of the somatotrophs have been reported (Carillo *et al.*, 1981) but the relation of this observation to circulating levels of GH is not known. In a preliminary study, Cook(1981) could find no evidence of a daily variation in serum levels of GH in goldfish, although only a limited number of environmental conditions were investigated, and it was suggested that GH was released in a pulsatile manner, similar to the situation in mammals. Other information on possible daily cycles in circulating GH levels in teleost fishes is not available.

In the present study, changes in circulating levels of GH in goldfish during a complete 24 hour period were determined at various times throughout the year. Changes in circulating levels of GH in relation to the seasonal somatic growth cycle of the goldfish were also investigated. The present report represents the first comprehensive study of this nature in a teleost species.

B. MATERIALS AND METHODS

Experimental Animals

Goldfish, *Carassius auratus*, of the common or comet varieties, were purchased from Ozark Fisheries, Stoutland, Missouri or Grassyforks Fisheries Co., Ltd., Martinsville, Indiana several times throughout the year in 1982 and 1983. On arrival, the fish were placed in flow-through stock aquaria (1500 or 4800 litre) and maintained for seven to fourteen days at 12-15 °C on a simulated natural photoperiod (Edmonton) as an initial acclimation period. During this time, the fish were fed in excess with commercially prepared fish food (Ewos pellets) at least twice daily. The chemical composition of the food used in the present study is shown in Appendix I.

Experimental Maintenance Regimes

Following the initial acclimation period, three groups of goldfish of mixed sex were randomly selected from the stock aquaria and anaesthetized by immersion in 0.05% tricaine methanesulphonate (MS-222) until opercular movements had ceased. The first group of goldfish consisted of approximately 10 animals of each sex and, after gentle blotting on damp paper towelling to remove excess moisture, each fish was weighed to the nearest decigram. The length of each fish, as measured from the tip of the snout to the end of the scale-covering on the body, was also recorded. All fish in this group were then killed by spinal transection and the weight of gonadal tissues noted for each fish.

Following anaesthesia, goldfish in the other two groups were fin-clipped for individual identification, and the body weight and length recorded for each fish. On recovery from the anaesthetic, each of the two groups were placed in a 225 litre flow-through aquarium. The fish were then exposed to temperature and photoperiodic conditions simulating natural environmental conditions (Edmonton) appropriate for the time of year during which the experiment was conducted. The photoperiod was adjusted weekly to allow for natural increases or decreases in daylength. In addition, full lights on and total darkness were preceded by 45 minutes of decreased light levels simulating natural dawn and dusk conditions, respectively. The fish were fed *ad libitum* (approximately 5% body weight in food per day) with Ewos pellets using automatic feeders

designed to deliver five to eight grams of food every 30 minutes throughout the photophase. This experiment was repeated several times throughout the year; the environmental conditions used in each experiment are summarized in Table 2.1.

Sampling Procedures

After a number of days of exposure to the experimental conditions (see Table 2.1), groups of six to seven fish were randomly selected for sampling every two hours, commencing at 08:00 hr and continuing for a minimum of 24 hours. For Experiment 2.5, fewer fish were available and groups of fish were sampled every three hours instead of every two hours. All fish in each sample group were from the same aquarium; selection of the sample groups alternated between the two aquaria so that, following the removal of two successive sample groups from the same aqaurium, the next two groups were selected from the other aquarium. Throughout the scotophase, removal of the fish was aided by the use of a dim red light.

On removal from the aquarium, the fish were anaesthetized by immersion in MS-222. During the scotophase, the fish were anaesthetized in darkness, although subsequent sampling took place in the light. Individual fish were identified by the fin-clip markings, gently blotted on damp paper towelling, and the body weight and length recorded for each fish. Blood samples were then taken from each fish by inserting a 25 gauge needle attached to a 1 ml disposable syringe into the caudal vasculature and removing 0.3 to 1 ml of blood; each blood sample was transferred to a test tube, placed over chiiped ice, and allowed to clot for several hours. Following centrifugation at 13000g, the serum was collected from each sample, immediately frozen on dry ice,and stored at -30 ^{0}C for several weeks until assayed for GH. Sampling of each group was started approximately 5 minutes before the designated sampling time and was usually completed 10 to 15 minutes after removal of the fish from the aquarium. On completion of sampling, each fish was killed by spinal transection, and the weight of gonadal tissues recorded for each fish.

Table 2.1. Summary of experimental conditions used in Experiments 2.1 through 2.7.

Experiment	Date of[1] Sampling	Photoperiod[2]		°C±1	N[3]	Length of Experiment[4]
		Lights On	Lights Off			
2.1	Feb. 15	08:00	17:20	6	99	28
2.2	Mar. 22	06:05	18:15	6	96	28
2.3[5]	May 8	05:08	19:43	10	108	28
2.4	June 10	04:05	20:53	18	101	23
2.5	July 5	04:15	21:07	22	61	24
2.6	Aug. 25	06:00	19:30	25	84	32
2.7	Nov. 9	08:00	17:00	9	104	30

1 Date on which sampling commenced.
2 Lights on and lights off preceded by 45 minutes of low light levels.
3 Total number of fish sampled.
4 Days of exposure to experimental conditions.
5 Growth hormone data not available.

Growth Hormone Radioimmunoassay

Serum levels of GH were measured using the RIA described by Cook *et al.* (1983). This RIA was developed using GH purified from carp pituitaries and has been extensively validated for the measurement of circulating GH levels in goldfish (Cook, 1981; Cook *et al.*, 1983). All samples from each experiment were assayed in duplicate within the same RIA. The procedures for this RIA are described fully elsewhere (Cook, 1981;Cook *et al.*, 1983).

Calculation of Growth Rates

Instantaneous relative growth rates were calculated using the following formula:

$$G = \frac{(w_2 - w_1) \times 100}{w_1 \times (t_2 - t_1)}$$

where G represents the percent increase in body weight per day, and w_1 and w_2 represent the body weight (g) at times t_1 and t_2, respectively (Ricker, 1979).

A linear growth rate (LGR) was calculated from body lengths using a similar equation:

$$LGR = \frac{(l_2 - l_1) \times 100}{l_1 \times (t_2 - t_1)}$$

where LGR is the percent increase in body length per day, and l_1 and l_2 represent body lengths (cm) at times t_1 and t_2, respectively.

A change in body weight may also reflect a change in gonadal weight and, therefore, a growth rate was calculated using the following formula:

$$SGR = \frac{(sw_2 - sw_1) \times 100}{sw_1 \times (t_2 - t_1)}$$

where SGR is the somatic growth rate or the percent increase in somatic weight per day, and sw_1 and sw_2 represent somatic weight (g) at times t_1 and t_2, respectively.

In this study, somatic weight is defined as the total body weight minus the weight of the gonadal tissue. The weight of the gonad for each fish could not be determined at the start of the experiment. Therefore, the gonosomatic index (GSI) of each fish in the group sacrificed at the start of the experiment was calculated as follows:

$$GSI = \frac{\text{gonadal weight X 100}}{\text{total body weight}}$$

The average GSI for each sex in the group sacrificed initially was determined and an appproximate somatic weight for each fish at the start of the experiment (ws_1) calculated as follows:

$$ws_1 = w_1 - [w_1 \text{ X } (GSI_1/100)]$$

where w_1 is the intital total body weight of the fish, and GSI_1 is the average GSI from fish of the same sex sacrificed at the start of the experiment. An approximate somatic weight for each fish at the end of the experiment (ws_2) was also calculated using a similar equation:

$$ws_2 = w_2 - [w_2 \text{ X } (GSI_2/100)]$$

where w_2 is the total body weight of the fish at the end of the experiment, and GSI_2 is the average GSI for all fish of the same sex sacrificed at the end of the experiment.

Statistical Procedures

Growth hormone and growth rate data were normalized using a logarithmic transformation. Analysis of variance and Duncan's multiple range test ($p<0.05$; Steel and Torrie, 1960) were used to determine differences in serum GH levels on both a daily and seasonal basis, and differences in growth rates throughout the year. Sexual differences in serum GH levels were determined using Student's t-test ($p<0.05$) whereas the Mann-Whitney U-test ($p<0.05$) was used to determine sexual differences in growth rates (Snedecor and Cochran, 1980). All calculations, descriptive statistics and statistical procedures were done using the Statistical Package for the Social Sciences (SPSS) programs (Nie *et al.*, 1975) available through the University of Alberta computing system.

C. RESULTS

Daily Variations in Serum Growth Hormone Levels

Serum GH levels from goldfish sampled over a 26 hour period in February of 1983 (Experiment 2.1) are shown in Figure 2.1. A significant increase in serum GH levels was detected in fish sampled at 20:00 hr as compared to fish sampled at 02:00, 04:00, 06:00, 08:00, 08:00B[1], 10:00, 12:00, 14:00, and 22:00 hr. Goldfish sampled at 04:00 hr had significantly lower serum GH levels as compared to fish sampled at 10:00B, 14:00, 16:00, 18:00, and 20:00 hr. Serum levels of GH in fish sampled 24 hours apart at both 08:00 and 10:00 hr were not significantly different.

Figure 2.2 shows the serum GH levels of goldfish sampled over a 26 hour period in March of 1983 (Experiment 2.2). In this experiment, a significant increase in serum GH levels was detected in fish sampled at 20:00 hr as compared to fish sampled at 08:00, 10:00, 14:00 and 24:00 hr. Fish sampled at 08:00 hr had significantly lower serum GH levels than fish sampled at 02:00, 04:00, 06:00, 08:00B, 10:00B, 16:00, 18:00, 20:00 and 22:00 hr. Serum levels of GH in fish sampled at 24:00 hr were also significantly lower than the levels in fish sampled at 10:00, 16:00 and 20:00 hr. Serum GH levels in fish sampled at 08:00B hr were significantly higher than in fish sampled 24 hours earlier at 08:00 hr.

Serum GH levels in fish sampled over a 28 hour period in June of 1983 (Experiment 2.4) are shown in Figure 2.3. Serum GH concentrations in fish sampled at 24:00 hr were significantly lower than in fish sampled at 04:00, 06:00, 08:00B, 10:00B, 10:00, 12:00, and 14:00 hr. Fish sampled at 18:00 hr also had significantly lower serum GH levels than fish sampled at 10:00 and 10:00B hr. Growth hormone levels in serum from fish sampled at 08:00 and 10:00 hr 24 hours apart were not significantly different.

Figure 2.4 depicts serum GH levels in goldfish sampled over a 28 hour period in July, 1983 (Experiment 2.5). Goldfish sampled at 09:00 hr had significantly higher serum concentrations of GH than fish sampled at 06:00, 15:00, 18:00 and 24:00 hr. Serum levels of GH in fish sampled 24 hours apart at both 09:00 and 12:00 hr were not significantly different.

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[1]The letter 'B' indicates the second sample 24 hours later at this time

Figure 2.1. Serum growth hormone (GH) levels from goldfish sampled in February, 1983. The fish were acclimated to 6 ± 1 ° C and a photoperiod simulating natural conditions(Edmonton). Lights on at 08:00 hr and lights off at 17:20 hr were preceded by 45 minutes simulating dawn and dusk conditions, respectively. Values are $\bar{X}$±SEM. The results of Duncan's multiple range test ($p<0.05$) are indicated; groups with common underscoring are not significantly different. For samples taken 24 hours apart, the second sample at a given time is represented by a closed triangle; in the range test results, the time followed by a "B" represents the second sample at that time. Note the $\log_{10}$ ordinate axis.

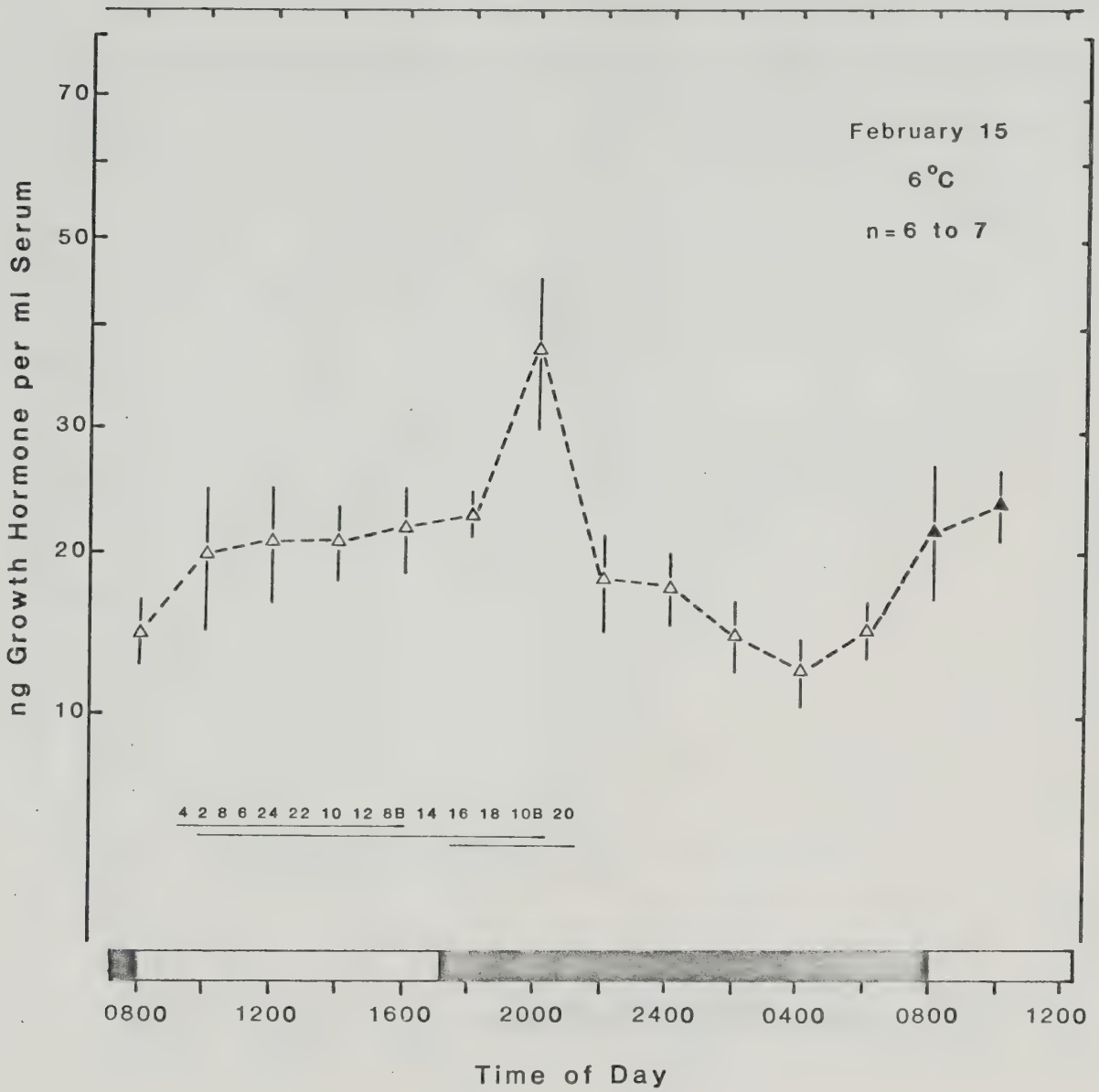


Figure 2.2. Serum growth hormone (GH) levels from goldfish sampled in March, 1983. The fish were acclimated to 6 ± 1 ° C and a photoperiod simulating natural conditions(Edmonton). Lights on at 06:05 hr and lights off at 18:15 hr were preceded by 45 minutes simulating dawn and dusk conditions, respectively. Values are $\bar{X}$±SEM. The results of Duncan's multiple range test ($p<0.05$) are indicated; groups with common underscoring are not significantly different. For samples taken 24 hours apart, the second sample at a given time is represented by a closed triangle; in the range test results, the time followed by a "B" represents the second sample at that time. Note the $\log_{10}$ ordinate axis.

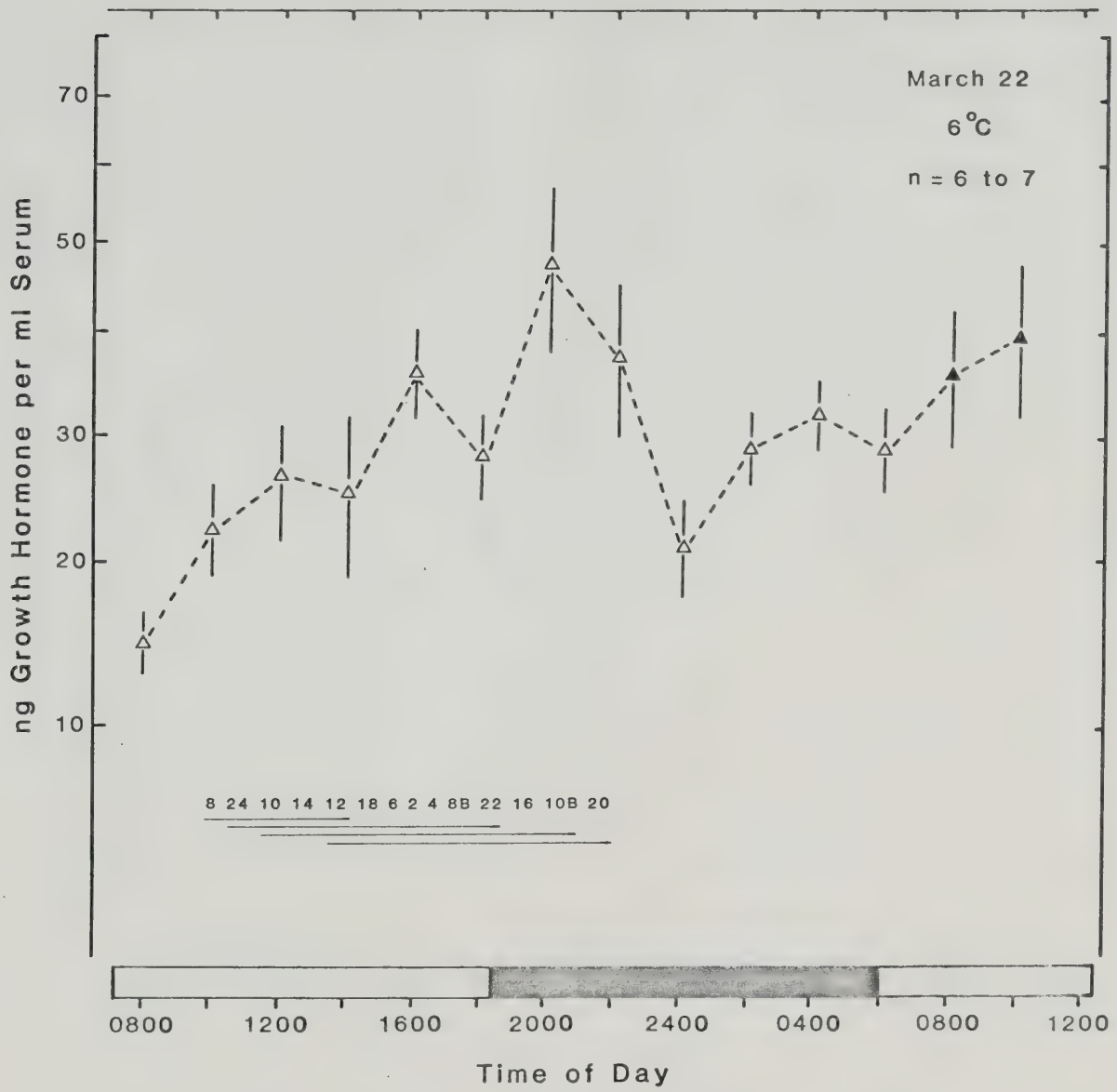


Figure 2.3. Serum growth hormone (GH) levels from goldfish sampled in June, 1983. The fish were acclimated to 18 ± 1 ° C and a photoperiod simulating natural conditions(Edmonton). Lights on at 04:05 hr and lights off at 20:53 hr were preceded by 45 minutes simulating dawn and dusk conditions, respectively. Values are $\bar{X}$±SEM. The results of Duncan's multiple range test ($p<0.05$) are indicated; groups with common underscoring are not significantly different. For samples taken 24 hours apart, the second sample at a given time is represented by a closed triangle; in the range test results, the time followed by a "B" represents the second sample at that time. Note the $\log_{10}$ ordinate axis.

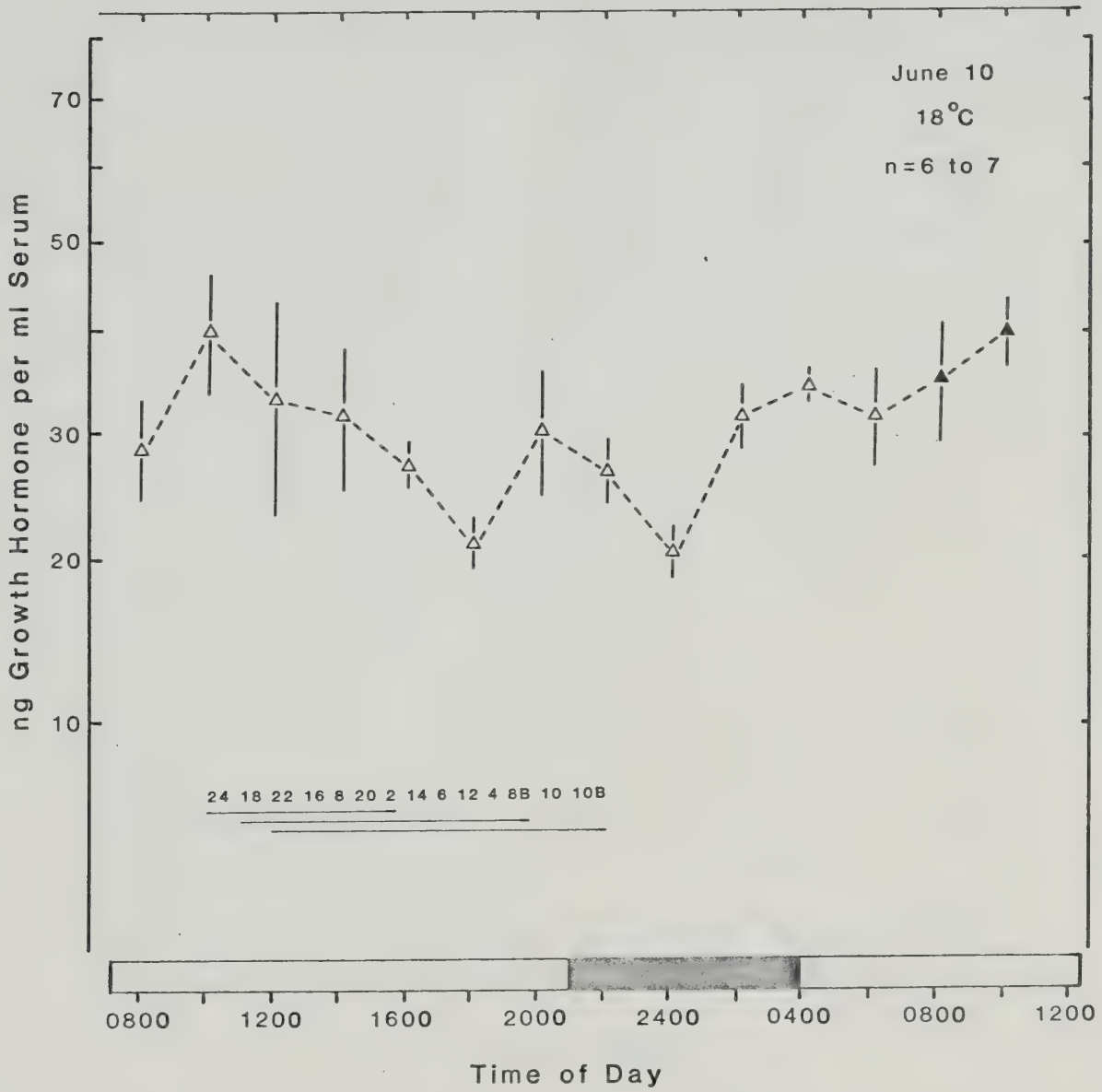
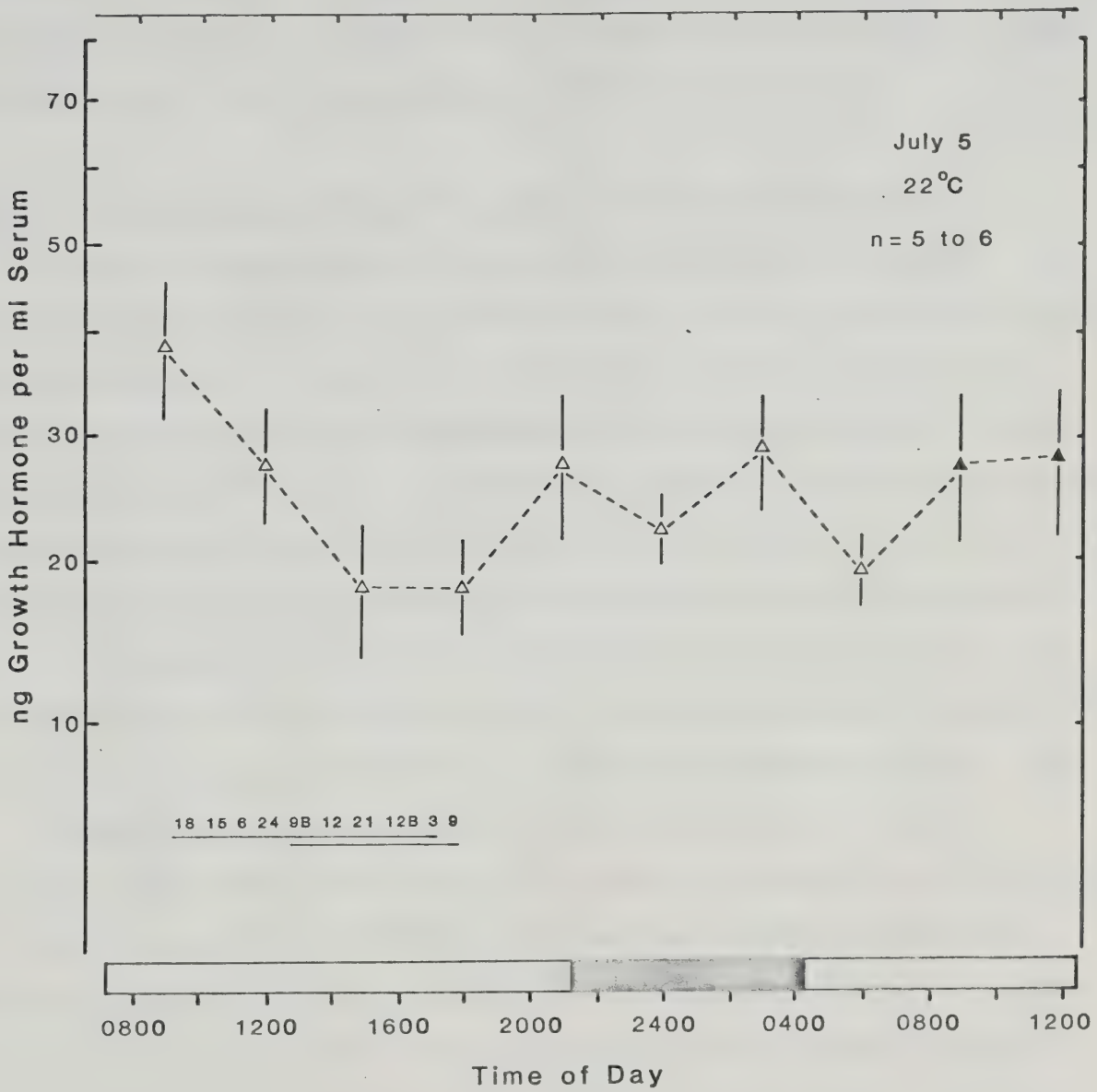


Figure 2.4. Serum growth hormone (GH) levels from goldfish sampled in July, 1983. The fish were acclimated to 22 ± 1 ° C and a photoperiod simulating natural conditions(Edmonton). Lights on at 04:15 hr and lights off at 21:07 hr were preceded by 45 minutes simulating dawn and dusk conditions, respectively. Values are $\bar{X}$±SEM. The results of Duncan's multiple range test ($p<0.05$) are indicated; groups with common underscoring are not significantly different. For samples taken 24 hours apart, the second sample at a given time is represented by a closed triangle; in the range test results, the time followed by a "B" represents the second sample at that time. Note the $\log_{10}$ ordinate axis.



In Figure 2.5 serum concentrations of GH in goldfish sampled over a 24 hour period in August, 1982 (Experiment 2.6) are shown. Fish sampled at 12:00 and 14:00 hr had significantly lower serum GH levels than fish sampled at 02:00 and 20:00 hr. Fish sampled 24 hours apart at 08:00 hr did not have significantly different serum GH levels.

Serum levels of GH in fish sampled over a 28 hour period in November of 1982 (Experiment 2.7) are shown in Figure 2.6 . Fish sampled at 02:00 hr had significantly lower serum levels of GH than fish sampled at 24:00 hr. Fish sampled 24 hours apart at 08:00, 10:00, and 12:00 hr did not have significantly different serum GH levels.

Seasonal Changes in Serum Growth Hormone Levels and Growth Rates

Variations throughout the year in serum GH levels are shown in Figure 2.7. The mean serum GH levels reported for the different times of the year were calculated from the values found over a 24 hour period in each of Experiments 2.1 through 2.7. The lowest mean daily serum GH level was measured in fish sampled in November; the serum GH level at this time of the year was significantly lower than at any other time of the year. The mean daily serum GH level in fish sampled in February was similar to the level found in goldfish sampled during August, and was significantly lower than the serum levels of GH found in fish sampled during March, June, and July. The highest circulating levels of GH were found in fish sampled in March and June; in fish sampled during July, the mean daily serum level of GH was significantly lower compared to fish sampled in March or June.

Changes in growth rates throughout the year are summarized in Table 2.2. Variations in SGR are also shown in Figure 2.7. The lowest SGR values were observed in fish sampled during February and March. In fish sampled during May, the SGR was significantly increased compared to the SGR of fish sampled earlier in the year, but the SGR in May was not significantly different from the SGR of fish sampled in November. Somatic growth rates were increased even more in fish sampled during June and July as compared to May; the SGR value in fish sampled in July was greater than in any other group. Goldfish sampled in August had a significantly lower SGR compared to fish in June or July. However, the SGR value during August was still significantly elevated as compared to the SGR at other times of the year. Relative changes throughout the year in the growth rate based on total body weight were similar to the seasonal variations in SGR (data not

Figure 2.5. Serum growth hormone (GH) levels from goldfish sampled in August, 1982. The fish were acclimated to 25 ± 1 ° C and a photoperiod simulating natural conditions(Edmonton). Lights on at 06:00 hr and lights off at 19:30 hr were preceded by 45 minutes simulating dawn and dusk conditions, respectively. Values are $\bar{X}$±SEM. The results of Duncan's multiple range test ($p<0.05$) are indicated; groups with common underscoring are not significantly different. For samples taken 24 hours apart, the second sample at a given time is represented by a closed triangle; in the range test results, the time followed by a "B" represents the second sample at that time. Note the $\log_{10}$ ordinate axis.

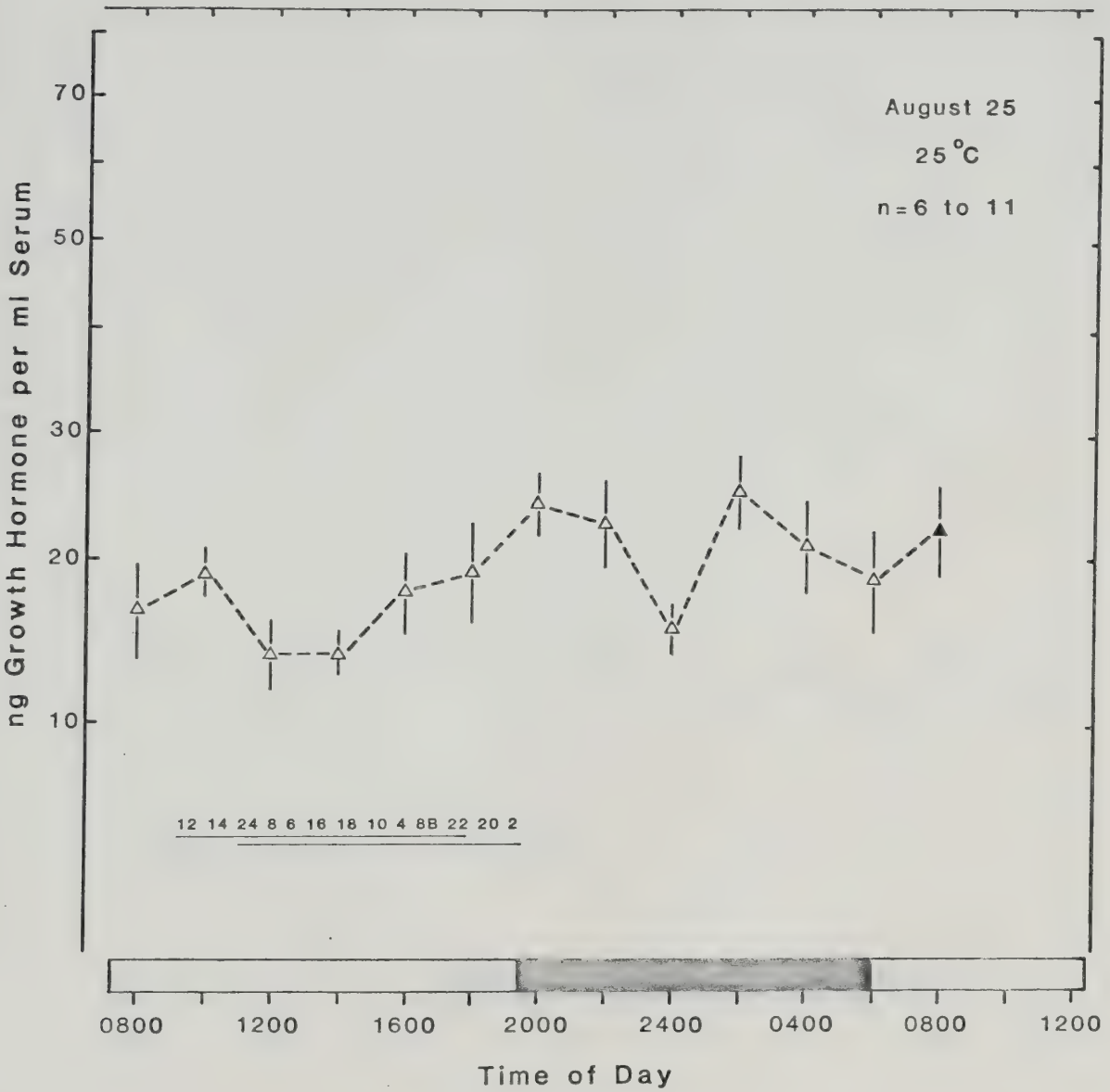


Figure 2.6. Serum growth hormone (GH) levels from goldfish sampled in November, 1982. The fish were acclimated to 9 ± 1 ° C and a photoperiod simulating natural conditions(Edmonton). Lights on at 08:00 hr and lights off at 17:00 hr were preceded by 45 minutes simulating dawn and dusk conditions, respectively. Values are $\bar{X}$±SEM. The results of Duncan's multiple range test ($p<0.05$) are indicated; groups with common underscoring are not significantly different. For samples taken 24 hours apart, the second sample at a given time is represented by a closed triangle; in the range test results, the time followed by a "B" represents the second sample at that time. Note the $\log_{10}$ ordinate axis.

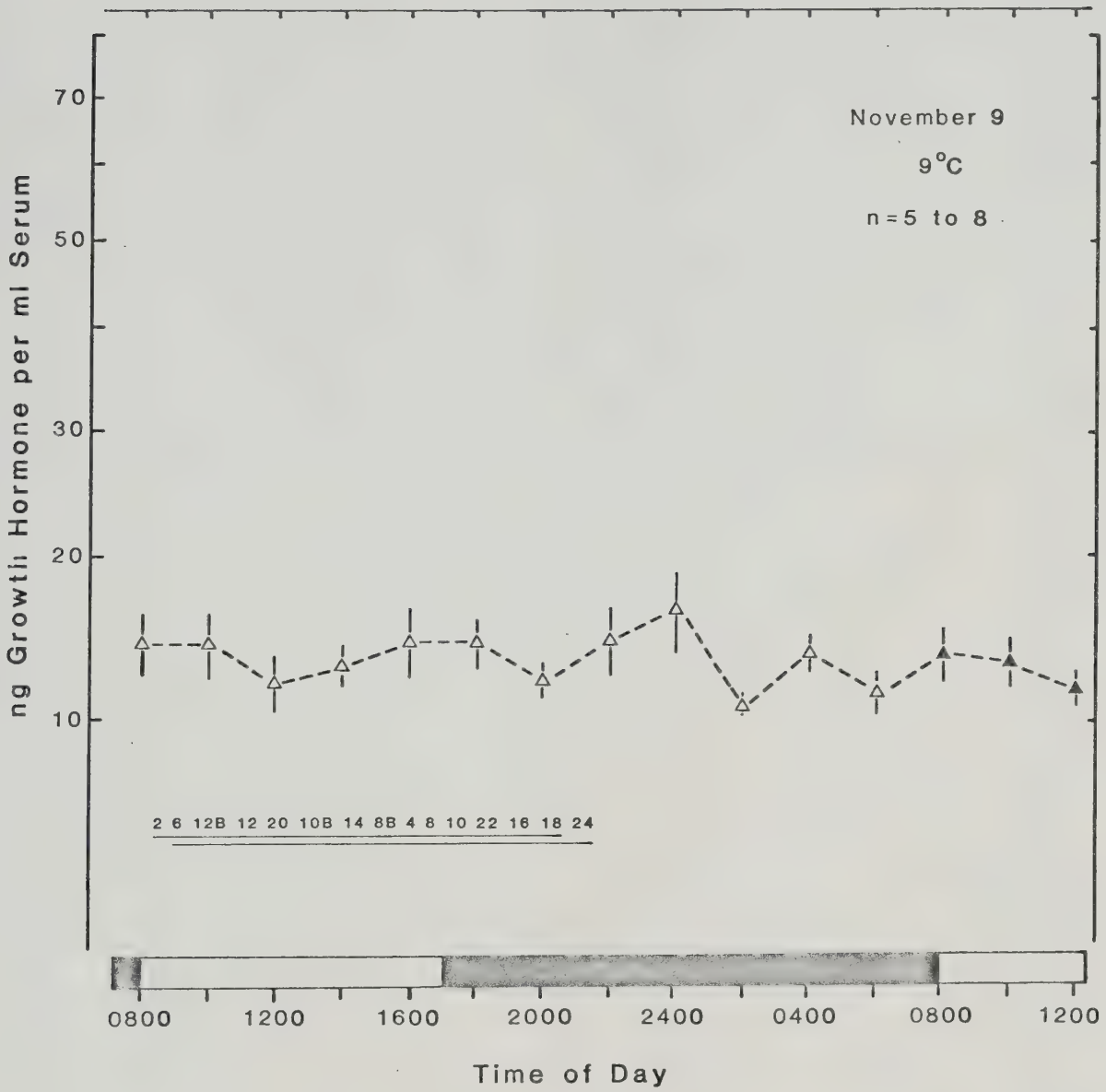


Figure 2.7. Seasonal variations in mean daily serum growth hormone (GH) levels (closed triangles) and somatic growth rates (SGR; open triangles) in goldfish. All values are represented as $\overline{X}\pm SEM$. The results of Duncan's multiple range test are indicated ($p<0.05$); groups with common underscoring are not significantly different. Note the $\log_{10}$ left ordinate axis.

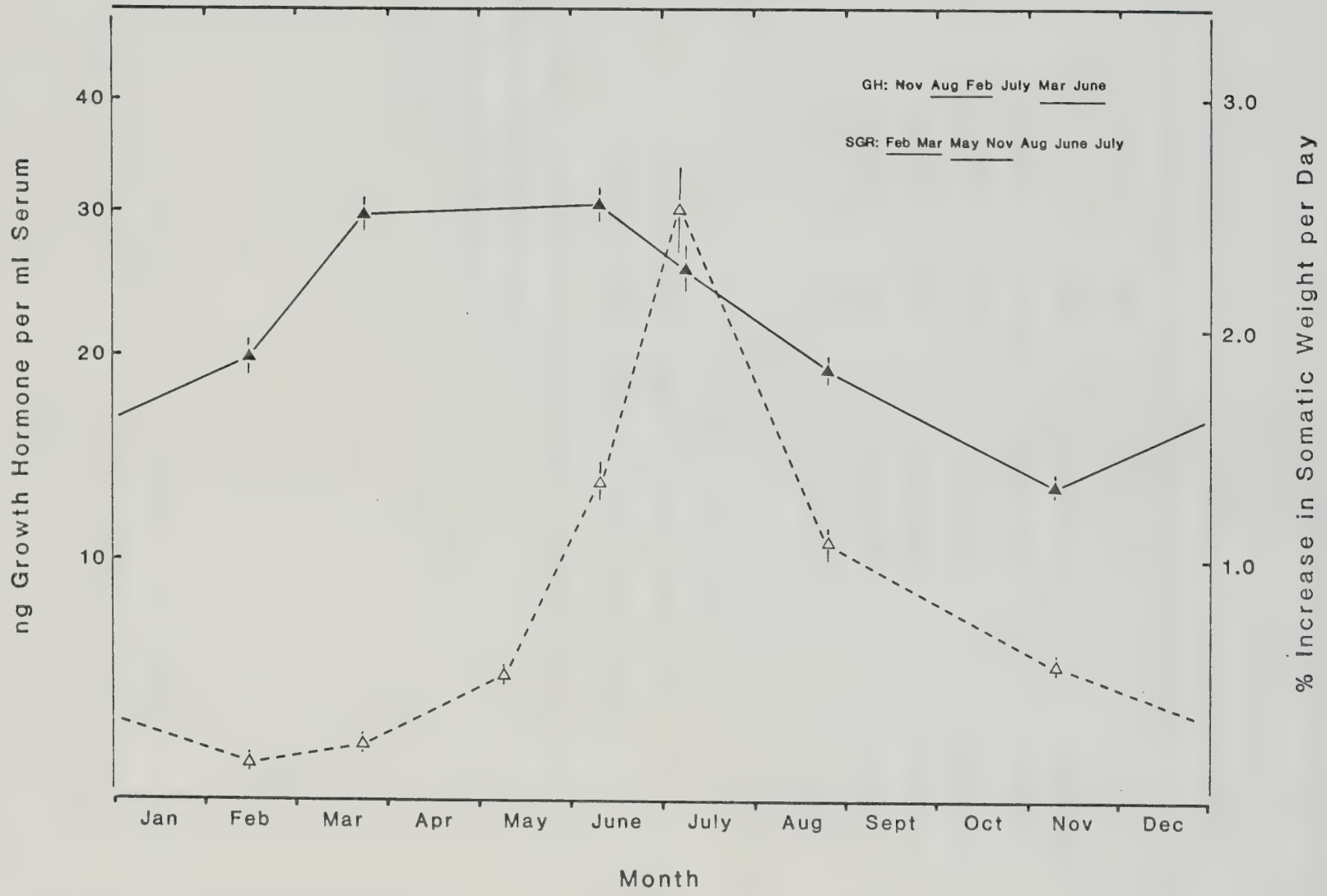


Table 2.2. Instantaneous relative growth rates from goldfish sampled at various times throughout the year.

			Instantaneous Relative Growth Rates	
Experiment	Date	N	SGR[1]	LGR[2]
2.1	Feb. 15	99	0.12±0.01[3]	0.07±0.01
2.2	Mar. 22	96	0.21±0.02	0.03±0.01
2.3	May 8	108	0.51±0.02	0.05±0.01
2.4	June 10	101	1.34±0.09	0.27±0.02
2.5	July 5	61	2.52±0.19	0.39±0.03
2.6	Aug. 25	84	1.07±0.07	0.17±0.03
2.7	Nov. 9	104	0.56±0.03	0.11±0.01

Duncan's Multiple Range Tests ($p<0.05$):

SGR: Feb Mar May Nov Aug June July

LGR: Mar May Feb Nov Aug June July

[1] % increase in somatic weight per day.

[2] % increase in body length per day.

[3] All values are $\bar{X}$±SEM.

shown).

The linear growth rate (LGR) was lowest in fish sampled in February, March and May; the mean LGR values at these times of the year were not significantly different (Table 2.2). In fish sampled during June and July, LGR values were significantly increased as compared to the earlier samples; the LGR value from fish in the July sample was higher than at any other sample time. The mean LGR value was significantly lower in fish sampled in August as compared to the June and July samples. In November the LGR was significantly decreased compared to fish sampled in August but the mean LGR value was still significantly greater than in fish sampled during February, March or May.

The groups of fish sampled in Experiments 2.1 through 2.7 contained both males and females (see MATERIALS and METHODS). In order to determine if sexual differences were present, serum GH levels, and the instantaneous relative growth rates were found for each sex at the various times throughout the year. The mean daily serum GH level, SGR, LGR, and GSI for each sex are presented in Table 2.3. The fish sampled in August were sexually regressed and the sex of each fish could not be visually determined; consequently, data for each sex at this time are not available. In Experiments 2.1 through 2.7, sample sizes for each sex were too small to allow for the statistical comparison of differences in serum GH levels between male and female goldfish at each of the times during the 24 hour sampling period. However, when the data for each 24 hour sampling period is pooled, the mean serum GH level did not differ significantly between the sexes in the experiments conducted from February to July (Table 2.3). On the other hand, in November, male goldfish had a significantly lower serum level of GH than female goldfish. Despite this difference, variations throughout the year in serum GH levels for each sex followed the trends observed when mean daily serum GH levels were averaged for both sexes (Figure 2.7).

Sexual differences in SGR were not observed in the experiments conducted in February and July. In the March, May, June and November experiments, however, female goldfish had a significantly higher SGR than male goldfish. In addition, during the June experiment, female goldfish had a significantly higher LGR than male goldfish; sexual differences in LGR were not observed at any other time of the year. Although sexual differences in growth rates were observed at certain times of the year, SGR and LGR for

Table 2.3. Mean daily serum growth hormone levels and instantaneous relative growth rates in male and female goldfish sampled at various times throughout the year.

Date of Experiment	Sex	N	GH (ng/ml)	Instantaneous Relative Growth Rates		GSI
				SGR[1]	LGR[2]	
Feb. 15	M[3]	44	19.3±1.9[4]	0.15±0.02	0.08±0.01	3.17±0.09
	F	53	20.5±1.2	0.10±0.02	0.07±0.01	7.78±0.45
Mar. 22	M	37	28.3±3.0	0.16±0.02[5]	0.03±0.01	3.99±0.19
	F	57	31.1±1.7	0.25±0.02	0.03±0.01	11.17±0.44
May 8	M	48	–	0.44±0.03[5]	0.04±0.01	3.65±0.13
	F	59	–	0.56±0.03	0.05±0.01	12.67±0.47
June 10	M	36	29.8±2.4	0.84±0.08[5]	0.14±0.02[5]	4.18±0.19
	F	57	30.8± 1.5	1.70±0.12	0.37±0.03	8.43±0.75
July 5	M	9	22.2±4.1	2.18±0.28	0.35±0.04	1.25±0.19
	F	48	26.2±1.8	2.58±0.21	0.40±0.04	2.24±0.45
Aug. 25	–	84	19.1±0.8	1.07±0.07	0.17±0.03	0.39±0.04
Nov. 9	M	46	11.8±0.5[5]	0.47±0.04[5]	0.10±0.01	1.32±0.09
	F	56	14.3±0.6	0.63±0.04	0.12±0.01	2.34±0.13

1 % increase in somatic weight per day.
2 % increase in body length per day.
3 M = male; F = female.
4 All data are $\bar{X}$±SEM.
5 Significantly different from female group ($p<0.05$).

both sexes followed the pattern observed when growth rates of both sexes were averaged (Table 2.2), with the highest growth rates occurring in June and July and reduced growth rates occurring in fish sampled in February or March.

D. DISCUSSION

In a preliminary study, Cook (1981) found no evidence for daily variations in serum GH levels in goldfish; the present study also provides little evidence for daily changes in serum GH levels throughout most of the year. In February, however, serum GH levels do fluctuate on a daily basis. At this time of the year, serum GH concentrations remain relatively stable throughout the photophase but increase substantially shortly after the start of the scotophase (Figure 2.1); thereafter, serum GH levels decrease and by the middle of the scotophase, serum GH levels have reached the lowest levels observed. By the start of the next photophase, serum GH levels have returned to values similar to those observed during the previous light phase. At this time of the year, therefore, there appears to be a peak in circulating GH levels that may be determined by the start of the scotophase or the end of the photophase.

In contrast to the February experiment, similar results were not obtained in any of the other experiments conducted at various times throughout the year. During the March experiment, peak serum GH levels were also attained shortly after the start of the scotophase (Figure 2.2), but serum GH levels from fish sampled 24 hours apart were significantly different. Due to the lack of duplication in serum GH concentrations, it is not likely that the pattern in serum GH levels in March is part of a regular daily rhythm. Although there were significant differences in serum GH levels between sample times within the 24 hour sampling period in the June, July and August experiments, there was also a lack of duplication in the serum GH measurments over the next 24 hour period in several instances (Figures 2.3 to 2.5), indicating that reproducible daily rhythms are not likely present at these times of the year. During November, the variability at each sample time is smaller but serum GH levels remained relatively unchanged throughout the 28 hour sampling period. Therefore, in the experiments conducted from March to November, there does not appear to be significant daily variations in circulating GH levels in goldfish.

In mammals, various stresses have been shown to alter GH release (for review: Chiodini and Liuzzi, 1979). In the present study, selection of the sample groups was interchanged between two aquaria, and it is possible that stresses associated with the removal of a sample group may have influenced the serum GH levels of the goldfish remaining in the aquarium. However, the magnitude and direction of the changes in serum

GH levels between the sample groups was different in Experiments 2.1 through 2.7, and would suggest that variations in serum GH levels observed throughout the sampling periods were not affected to any large extent by the sampling procedure. Furthermore, Cook (1981) could find little evidence to suggest that stresses associated with blood sampling influenced subsequent serum GH levels in goldfish. On this basis, it is not likely that stresses associated with sampling are responsible for the results obtained in Experiments 2.1 through 2.7; however, the use of groups of goldfish maintained in individual aquaria would have reduced the possibility of stress-induced changes in serum GH levels.

In other teleost species, there is only circumstantial evidence to suggest the presence of a daily rhythm in circulating GH levels. In one study using kokanee salmon, significant peaks in plasma GH levels were observed at 03:00 and 12:00 hr in juvenile fish maintained at 10-12 °C under a natural photoperiod (Leatherland *et al.*, 1974). However, these authors did not present evidence concerning the reproducibility of this rhythm, or whether similar variations were present at other times of the year or under other environmental conditions. The validity of the heterologous RIA used in the study is also questionable (Nicoll, 1975). A daily variation in the nuclear area of somatotrophs in tilapia has also been demonstrated (Carillo *et al.*, 1980). In tilapia, the nuclear area of the somatotrophs was greatest at the end of the photophase and at eight hours after the start of the scotophase. However, the relationship between daily changes in the nuclear area of somatotrophs and circulating levels of GH in the tilapia is not known.

Further research is required to investigate the possibility that daily rhythms in serum GH levels may be present in goldfish maintained under other experimental conditions. For example, a number of physiological processes are known to be influenced by food intake (Delahunty *et al;*, 1978). In trout (*Salmo gairdneri*), daily rhythms in thyroid hormones have also been shown to be influenced by food intake (Eales *et al.*, 1981). The pulsatile release of GH in rats is known to be suppressed by food deprivation (Tannenbaum, 1981), and it is possible that food intake may also influence serum GH levels in goldfish. In the present study, food was available continuously throughout the photophase. Further research is required to determine if feeding on a more restricted ,but regular basis, may influence secretion of GH in the goldfish.

Seasonal variations in the growth rate of somatic tissues have been described for a wide variety of teleost species (see INTRODUCTION), and a number of studies have suggested that changes in circulating levels of GH may be associated with the seasonal cycle in growth rates (Pickford, 1957; Gross *et al.*, 1965; Swift and Pickford, 1965; Gerking, 1966; Shul'man, 1974; Cook, 1981). The present study, however, is the first to describe serum levels of GH associated with the seasonal variations in growth rates in a teleost species. In the goldfish, the highest mean daily serum levels of GH were found in goldfish sampled in March and June, whereas the lowest level occurred in fish sampled in November. The highest mean daily serum levels of GH occurred several weeks before significant increases in the growth rates were found; a significant increase in the somatic growth rate was not detected until May whereas a significant acceleration in the linear growth rate was not observed until June. In goldfish, therefore, the seasonal increase in circulating GH levels precedes the seasonal increase in growth rates.

In a study using hypophysectomized *Fundulus heteroclitus* to bioassay the GH content of the pituitary glands of perch, *Perca fluviatilis*, captured at various times of the year, Swift and Pickford (1965) found that the growth-promoting activity of perch pituitaries was highest in June, one month prior to the period of maximum growth in perch. The observation that, in goldfish and presumably perch, the seasonal peak in circulating GH levels occurs several weeks prior to the optimal growth rate of the fish may be related to a number of parameters. Swift and Pickford (1965) suggested that water temperature may be important in governing two aspects of the seasonal cycle in GH secretion. Firstly, increasing water temperatures during the spring may stimulate increased production and secretion of GH; secondly, increased temperature may also increase the responsiveness of target tissues to GH. In the present study, the increase in mean daily serum GH levels in March cannot be explained by temperature-induced increases in the production and secretion of GH by the pituitary, because the water temperature in March was identical to the temperature used in the February experiment (6 ^{0}C). Furthermore, in June, the mean daily serum GH levels were similar to those found in March although the temperature had increased to 18 ^{0}C.

On the basis of their data, Swift and Pickford (1965) could not rule out, or support, the possibility that increasing daylengths during the spring months may stimulate the

seasonal increase in circulating GH levels. In the present study, increases in serum GH levels from February to June correspond well with the increasing daylengths, whereas from July to November, serum levels of GH decrease as the daylength becomes shorter. Although this evidence is only circumstantial, variations in the duration of the photophase or scotophase throughout the year may serve as the environmental cue by which seasonal variations in mean daily circulating GH levels are regulated.

Environmental temperature has been shown to influence the growth of many teleost species (for review: Brett, 1979). In most fishes, the growth rate increases with increasing water temperature until an optimal temperature is reached, above which growth rates decline. Temperature is known to affect various parameters related to the growth of fishes, including food availability and intake, metabolic requirements, and food conversion rates and efficiencies (Shul'man, 1974; Brett, 1979). It has also been suggested that temperature may influence the secretory dynamics of GH and the responsiveness of tissues to GH (Swift and Pickford, 1965; Adelman, 1977; Kayes, 1977). In the study by Swift and Pickford (1965) it was suggested that the delay between maximal pituitary GH concentrations and maximum growth rates was a result of reduced target–tissue responsiveness at lower water temperatures. This hypothesis may also explain the the lag period between maximal serum GH concentrations and growth rates observed in goldfish.

In the present study, the increases in growth rate were closely related to changes in water temperature. A significant acceleration in the somatic growth rate was not observed until May when water temperature had increased from 6 to 10 °C; a significant increase in the linear growth rate was not detected until June when the water temperature was 18 °C. The highest growth rates were attained in July, when water temperature was 22 °C, several weeks after the peak in mean daily serum GH concentrations had been reached. In August, temperature was increased to 25 °C but the growth rates had declined by this time. It is not likely that the optimum temperature for growth in goldfish had been exceeded in August, as 25 °C is within the limits for optimal growth of carp (Adelman, 1977), and the decline in growth rates in August is most likely related to reduced circulating levels of GH at this time. In November, growth rates declined further as temperature was reduced to 9 °C; the lowest serum levels of GH were detected at this

time although the lowest growth rates were not observed until several weeks later when water temperatures were lowest. Therefore, in goldfish, there is a delay between maximum serum GH levels and growth rates and minimum growth rates and serum GH levels.

The level at which temperature may act to reduce tissue responsiveness is not known. In addition to the various metabolic processes listed above, temperature would presumably influence various other parameters related to growth or the action of GH, including GH receptors, the rate of protein synthesis and bone growth, the metabolic clearance rate of GH from the circulation, or other hormones such as the thyroid hormones, which may act synergistically with GH to promote growth. In mammals, the actions of GH are known to be mediated by a class of secondary hormonal agents known as the somatomedins (Daughaday, 1982). Although the presence of somatomedins in teleost fishes has not been satisfactorily demonstrated , it is interesting to speculate that temperature may also affect various aspects related to the metabolic activity of these somatomedins. Obviously, more research is required before the mechanism by which temperature influences growth rates in teleost fishes is known.

In the present study, significant differences in growth rates between male and female goldfish were found (Table 2.3). A number of other teleost species have been reported to exhibit sexual variations in growth rates (Shul'man, 1974; Hunt and Jones, 1975). Although female goldfish did have faster growth rates at certain times of the year, the pattern of seasonal variations in growth rates was similar for both sexes, justifying the combination of data from both sexes for statistical comparison and discussion in the previous paragraphs.

Significant sexual differences in serum levels of GH were not detected in fish sampled from February to July but, in November, female fish did have a significantly higher mean daily serum GH level as compared to male fish. However, the growth rates were slower at this time, and the serum GH levels of fish sampled in November were the lowest detected throughout the year, suggesting that the sexual difference in the serum GH level may not be physiologically significant

In summary, significant daily fluctuations in the serum GH level in goldfish appear to be absent throughout most of the year; in February, however, a significant daily variation is

present. In both male and female goldfish, mean daily serum GH levels are highest from March through June, and lowest in November. It is suggested that seasonal changes in the duration of the photophase or scotophase may act as the environmental factor by which seasonal changes in mean daily serum GH levels are regulated. There are also seasonal variations in growth rates in goldfish that are similar to those reported for other temperate–zone teleost fishes. A lag period of several weeks between seasonal maxima in mean daily serum GH levels and growth rates is most likely due to reduced metabolic activity of GH as a result of lower water temperatures; as water temperatures increase, the metabolic activity of GH also increases. Although female goldfish exhibit faster growth rates than male fish at certain times of the year, these variations do not appear to be related to sexual differences in serum GH levels.

III. INFLUENCES OF TEMPERATURE AND PHOTOPERIOD ON THE RATE OF BODY GROWTH, AND ON CIRCULATING GROWTH HORMONE LEVELS IN GOLDFISH, *Carassius auratus*

A. INTRODUCTION

Temperature and photoperiod are known to be two environmental variables with the potential to influence the rate of body growth in teleost fishes (Shul'man, 1974; Brett, 1979). Generally, growth rates have been shown to increase with increasing water temperature until an optimum temperature is reached, above which an increase in temperature results in decreased growth rates (Brett, 1979). Environmental temperature acts as a "Controlling Factor", influencing metabolic expenditures, food intake and conversion rates, and related metabolic processes which ultimately affect the rate of somatic growth in teleost fishes (Brett, 1979). In Chapter II, growth rates throughout the year in goldfish were closely related to changes in water temperature, with increased growth rates present in fish maintained at the warmer temperatures (Table 2.2). The influence of photoperiod on body growth in teleost fishes is not as well documented, although, in general, long daylength or increasing daylengths over a period of time is stimulatory to body growth in freshwater fishes, especially when applied at certain times of the year (Brett, 1979).

Although the effects of temperature and photoperiod on body growth have been studied in several teleost species, the influence of these two variables on endogenous levels of GH have not been documented. Growth response to exogenously administered GH have been reported to be temperature dependent in a number of teleost species (Pickford, 1957; Adelman, 1977; Kayes, 1977), and it has been suggested that temperature may influence endogenous levels of GH, metabolic turnover rates of GH, or target-tissue responsiveness to GH (Pickford, 1957; Swift and Pickford, 1965; Adelman, 1977; Kayes, 1977). In a preliminary study by Cook (1981), the mean daily serum GH level was increased in goldfish exposed to a water temperature of 20 °C in February, as compared to fish maintained at 12 °C; however, a similar response to temperature was absent in goldfish sampled during April. The effect of temperature on endogenous levels of GH at other times of the year and under other environmental conditions were not

studied.

The influence of photoperiodic conditions on secretion of GH has not been investigated, although light is thought to be a "Directive Factor" capable of stimulating brain–pituitary responses in teleost fishes (Brett, 1979). Swift and Pickford (1965) have suggested that seasonal increases in the natural daylength may act as the initial stimulus to hypophyseal GH synthesis in perch, *Perca fluviatilis*; in goldfish, seasonal variations in serum GH levels may also be related to natural changes in daylength (Chapter II). In contrast, photoperiodic conditions did not influence the growth response of common carp, *Cyprinus carpio*, to bovine GH injections (Adelman, 1977).

In the present study, the influences of various temperature and photoperiod conditions on the rate of somatic growth in goldfish were studied using a factorial design with two levels of constant temperature and daylength; this design allowed the influence of each environmental variable and the possible interaction of photoperiod and temperature to be evaluated. Serum GH levels in the fish exposed to the environmental regimes were also determined. This experiment was repeated several times throughout the year to determine if the influences of temperature and photoperiod on the rate of body growth in goldfish also vary on a seasonal basis.

B. MATERIALS AND METHODS

Experimental Animals

The source and maintenance procedures for the goldfish used in the present study were described previously (Chapter II).

Experimental Procedures

Following the initial acclimation period to general laboratory conditions, a group of 10 goldfish was randomly selected from the stock aquarium shortly before 08:00 hr. After anaesthetization in MS-222 and gentle blotting on damp paper towelling, the body weight and length were recorded for each fish and a blood sample removed from the caudal vasculature. The fish were then killed by spinal transection and the weight of gonadal tissues recorded. A second group of 10 goldfish was also removed from the stock aquarium at 16:00 hr of the same day and sampled in a similar manner. Sampling of these two groups was started approximately 10 minutes before the designated sampling time and was usually completed within 15 to 20 minutes of the removal of the fish from the aquarium.

After sampling of the 08:00 hr group was completed, eight additional groups of goldfish were randomly selected from the stock aquarium, with each group consisting of 20 goldfish of mixed sex (when possible, each group consisted of 10 male and 10 female goldfish). The fish were anaesthetized in MS-222, fin-clipped for individual identification, and the body weight and length recorded for each fish. On recovery from the anaesthetic, each group of goldfish was placed in an individual 90 litre flow-through aquarium, and acclimated for a period of eight days to a photoperiod of 12 hours light and 12 hours darkness (12L:12D) and a water temperature of 12±1 °C.

On the morning of the ninth day, designated as Day 1 of the experimental period, each group of fish was anaesthetized, and the body weight and length recorded for each fish. The fish were returned to the aquaria, and two groups randomly selected and exposed to each of the following conditions: 16L:8D, 12±1 °C; 16L:8D, 20±1 °C; 8L:16D, 12±1 °C; 8L:16D, 20±1 °C. For the groups exposed to 20 °C, the water temperature was slowly raised over a period of several hours. Although the time of lights on was

approximately 08:00 hr, the timing was staggered in the aquaria so that subsequent sampling of the experimental groups (see below) would occur at approximately the same time during the photoperiod. Throughout the acclimation period, the fish were fed in excess twice daily with Ewos pellets. The groups of fish sampled on Day 10 of the experimental period (see below) were also fed twice daily. However, the groups of goldfish sampled on Day 30 were fed *ad libitum* with Ewos pellets using the automatic feeders described previously (Chapter II).

On Day 10 of the experimental period, four groups of goldfish exposed to each of the environmental conditions were sampled. At 08:00 hr, one half of the fish in one of the groups was randomly selected from the aquarium and anaesthetized in MS-222. The body weight and length were determined for each fish, and a blood sample taken from the caudal vasculature. The fish were then killed by spinal transection and the gonadal tissues removed and weighed. This sampling was repeated until half of the goldfish in each of the four aquaria had been sampled at 08:00 hr. The goldfish remaining in these four aquaria were sampled in a similar manner at 16:00 hr of the same day. On Day 30 of the experimental period, these sampling procedures were repeated, using the goldfish in the remaining four aquaria.

Several experiments using the procedures described above were performed at various times throughout the year. A total of six experiments were conducted; the times of the year during which the experiments were performed and the gonosomatic index (GSI) of the fish sampled at the start of each experiment are shown in Table 3.1.

Blood Sampling Procedures

Blood samples were obtained from the caudal vasculature of the goldfish, and the serum from each sample collected and stored using the methods described previously (Chapter II).

Growth Hormone Radioimmunoassay

Serum levels of GH were determined using the RIA described briefly in Chapter II.

Table 3.1. The times of year during which Experiment 3.1 through 3.6 were conducted, and the gonosomatic index (GSI) of male and female goldfish sacrificed at the start of each experiment.

Experiment	Time of Year	Sex	N	GSI
3.1	January–February	M[1]	7	4.8±0.7[2]
		F	13	7.1±0.9
3.2	March–April	M	9	3.3±0.3
		F	11	9.2±1.2
3.3	April–May	M	10	5.8±0.5
		F	10	13.4±1.1
3.4	June–July	M	5	2.7±0.2
		F	15	7.7±0.9
3.5	September–October	–	10	0.4±0.1
3.6	November–December	M	5	1.8±0.2
		F	5	4.0±0.3

[1] M=male;F=female.
[2] All values are $\bar{X}$±SEM.

Calculation of Growth Rates

Instantaneous relative growth rates (SGR and LGR) were calculated for goldfish sacrificed on Day 30 only, using the equations described previously (Chapter II). Somatic weights on Day 1 were calculated using the average GSI from the group of fish sacrificed at the start of the experiment; on Day 30, somatic weights were calculated using the average GSI values from fish of the same sex sacrificed at this time.

Statistical Procedures

Growth hormone and growth rate data were normalized using a logarithmic transformation. The influence of temperature and photoperiod on serum GH levels on Day 10 and Day 30, and on the growth rates were analyzed using factorial analysis of variance ($p<0.05$; Snedecor and Cochran, 1980). In all groups, serum GH levels represent the average of the values obtained from the 08:00 and 16:00 hr samples. For the fish sacrificed on Day 10 and on Day 30, differences in the mean serum GH levels between the experimental groups were analyzed using one-way analysis of variance and Duncan's multiple range test ($p<0.05$; Steel and Torrie, 1960). Analysis of variance and Duncan's multiple range test ($p<0.01$) were also used to analyze differences in the growth rates between experimental groups on Day 30. Sample sizes of less than 20 resulted from the death of some of the animals during the experimental period; in the case of the growth rates, fish which could not be identified from the fin-clip markings were not included in the analysis.

Student's t-test ($p<0.05$) was used to test for sexual variations, differences between the 08:00 hr and 16:00 hr samples, and differences between the Day 10 and Day 30 samples in serum GH levels within each experimental group (Snedecor and Cochran, 1980). Sexual differences in the growth rates were analyzed using the Mann-Whitney U-test ($P<0.01$). All descriptive statistics, calculations, and statistical analyses were done using the SPSS (Nie *et al.*, 1975) and BMDP (Dixon *et al.*, 1981) program packages available through the University of Alberta computing system.

C. RESULTS

Experiment 3.1 (January-February)

Figure 3.1 shows the serum GH levels in the experimental groups from the January–February experiment. There were no significant differences in serum GH levels between the 08:00 and 16:00 hr samples, or between male and female goldfish in any of the experimental groups (data not shown). On both Day 10 and Day 30, the lowest serum GH level was detected in goldfish exposed to 8L:16D, 12 °C; the serum GH level in this group was similar to the serum GH level of fish exposed to 16L:8D, 12 °C but was significantly lower than the serum GH levels in all other groups. Fish subjected to 16L:8D, 20 °C and 8L:16D, 20 °C had similar serum GH levels; in both these groups, the serum GH levels were significantly higher as compared to each of the other groups.

Corresponding instantaneous relative growth rates (SGR and LGR) are shown in Table 3.2 and Table 3.3 . The SGR was significantly higher in the group of fish exposed to 16L:8D, 20 °C. The LGR showed a similar trend, with the highest rate present in the 16L:8D, 20 °C group. Significant sexual differences in the growth rates were not detected (data not shown).

The results of the factorial analysis of variance are summarized in Table 3.4. On both Day 10 and Day 30, only temperature significantly influenced serum GH levels. However, the interaction of temperature and photoperiod did have a significant effect on both SGR and LGR.

Experiment 3.2 (March-April)

Serum GH levels in Experiment 3.2 are shown in Figure 3.2 . In the 8L:16D, 20 °C group sacrificed on Day 10, fish sampled at 08:00 had a significantly increased serum GH level compared to fish sampled at 16:00 hr (59.0±8.8 and 32.2±1.2, respectively); on Day 30, the 08:00 hr sample in this group also had a significantly higher serum GH level than the fish sampled at 16:00 hr (77.6± 7.9 and 34.1±7.8, respectively). No other group had significant differences in serum GH levels between the 08:00 and 16:00 hr samples (data not shown). Also, no significant sexual differences in serum GH levels were observed within any experimental group (data not shown).

Figure 3.1. Serum growth hormone (GH) levels in goldfish maintained under different environmental regimes in January–February. All values are $\bar{X}\pm SEM$. The initial sample represents the serum GH level in fish sampled at the start of the experiment. The results of Duncan's multiple range test are indicated; groups with common underscoring are not significantly different ($p>0.05$). The bracketed letters below the abscissa represent each experimental group in the range test. The numbers above the abscissa are the sample size of each group. Note the $\log_{10}$ ordinate axis.

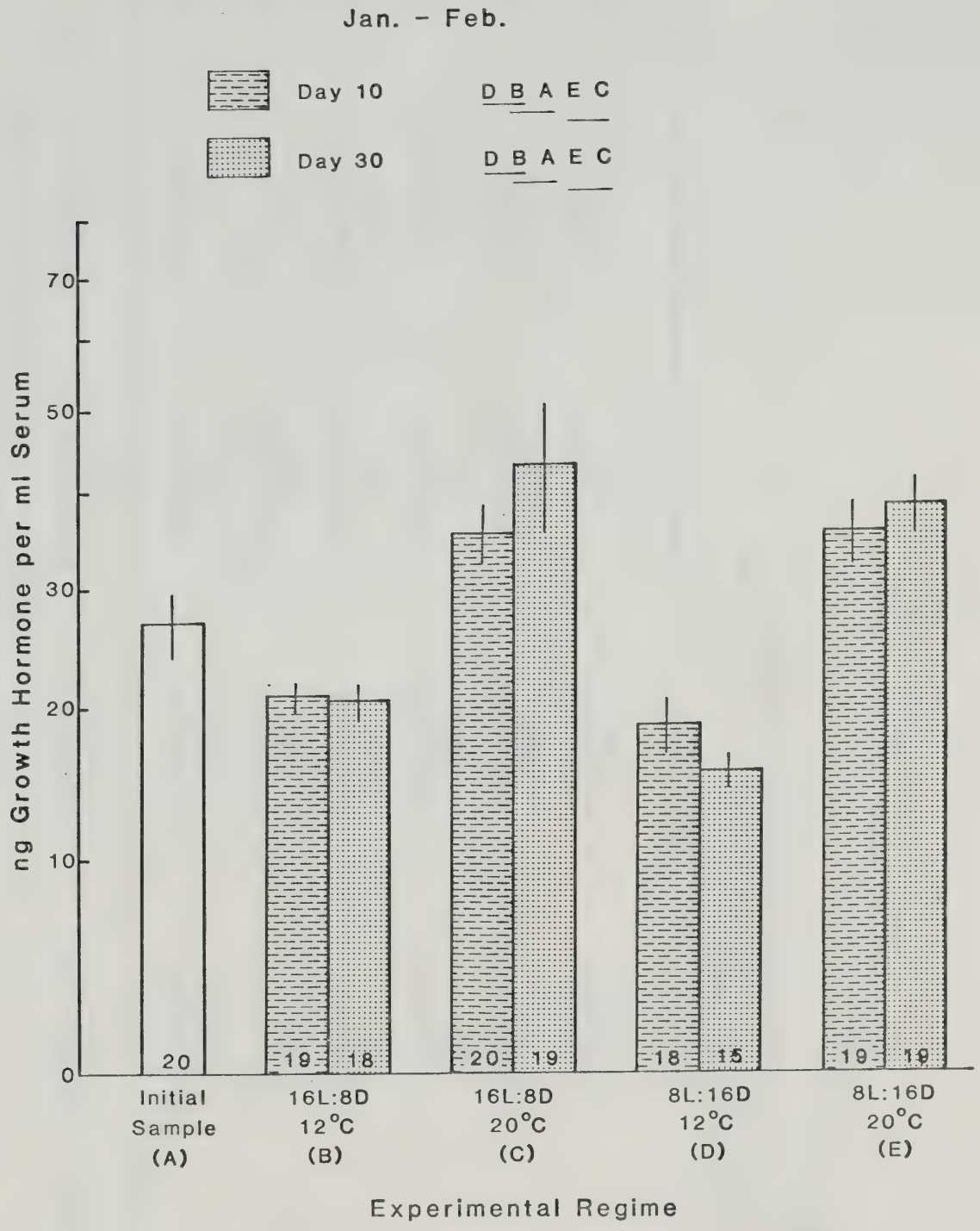


Table 3.2. Somatic growth rates of goldfish kept under different environmental regimes at various times throughout the year.

Experiment / Regime:	Somatic Growth Rates[1] 16L:8D, 12 °C (B)[2]	16L:8D, 20 °C (C)	8L:16D, 12 °C (D)	8L:16D, 20 °C (E)	Duncan's Multiple Range Test[3]
January–February	0.58±0.04[4] (17)[5]	1.43±0.13 (18)	0.55±0.07 (16)	0.63±0.09 (17)	D B E C
March–April	0.34±0.06 (12)	0.98±0.31 (10)	0.33±0.09 (12)	0.97±0.22 (9)	D B E C
April–May	1.03±0.07 (14)	1.46±0.09 (20)	0.88±0.07 (9)	0.86±0.06 (18)	E D B C
June–July	0.58±0.06 (10)	0.91±0.25 (10)	0.47±0.07 (9)	1.12±0.32 (8)	D B C E
September–October	0.60±0.05 (19)	1.89±0.20 (20)	0.75±0.04 (19)	2.28±0.11 (20)	B D C E
November–December	0.77±0.06 (20)	0.75±0.09 (19)	0.71±0.04 (18)	0.99±0.07 (19)	D C B E

[1] % increase in somatic weight per day.
[2] Letters in brackets represent the experimental groups in the range test.
[3] Groups with common underscoring are not significantly different.
[4] All data are $\bar{X}$±SEM.
[5] Numbers in brackets are sample sizes.

Table 3.3. Linear growth rates of goldfish kept under different environmental regimes at various times throughout the year.

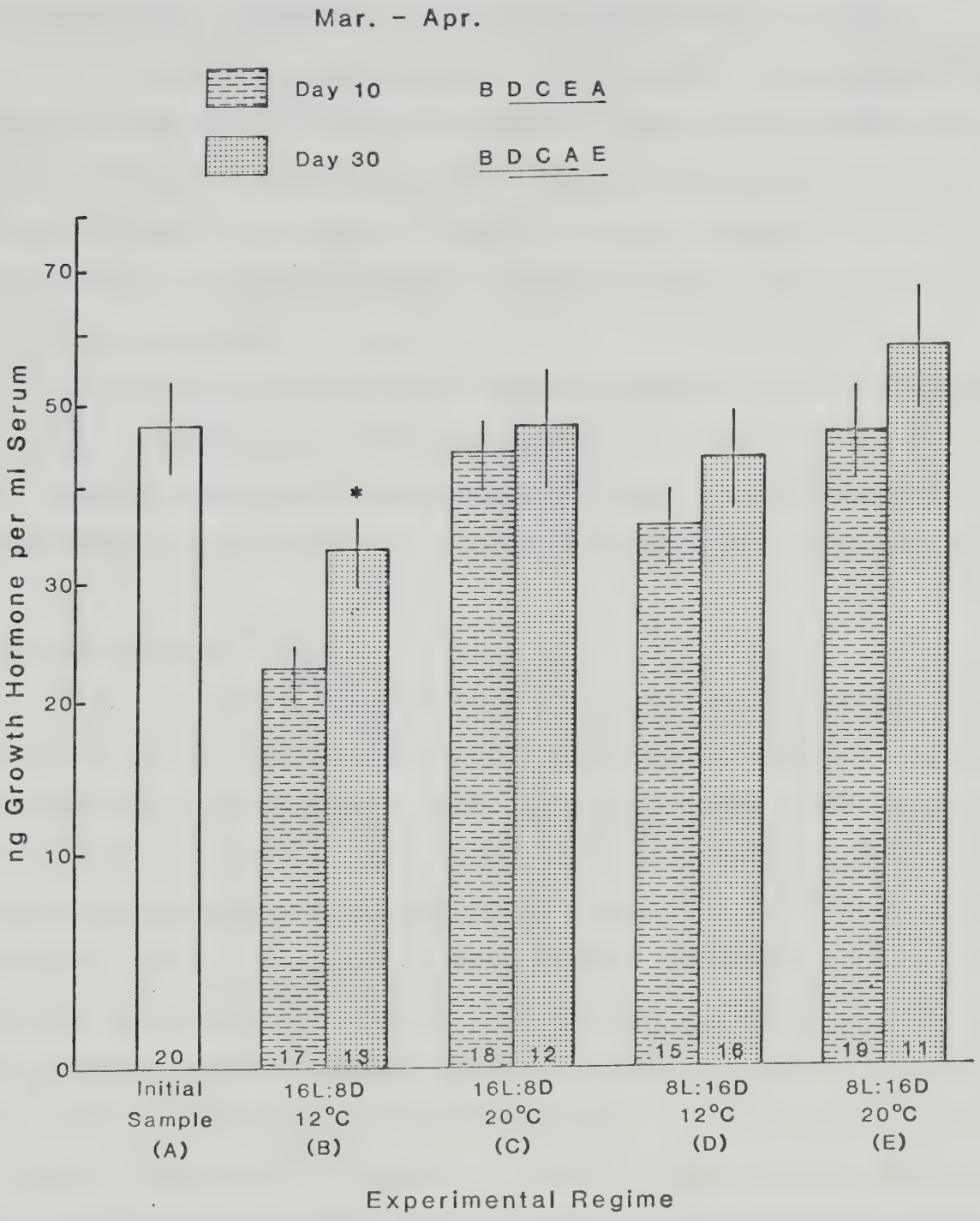
Experiment / Regime:	Linear Growth Rates[1] 16L:8D, 12 °C (B)[2]	16L:8D, 20 °C (C)	8L:16D, 12 °C (D)	8L:16D, 20 °C (E)	Duncan's Multiple Range Test[3]
January–February	0.09±0.01[4] (17)[5]	0.32±0.03 (18)	0.11±0.01 (16)	0.17±0.02 (17)	B D E C
March–April	0.07±0.01 (12)	0.18±0.03 (10)	0.05±0.01 (12)	0.13±0.05 (9)	D B E C
April–May	0.05±0.01 (14)	0.23±0.02 (20)	0.05±0.01 (9)	0.15±0.01 (18)	B D E C
June–July	0.08±0.02 (10)	0.19±0.06 (10)	0.06±0.01 (9)	0.17±0.06 (8)	D B E C
September–October	0.09±0.01 (19)	0.40±0.04 (20)	0.11±0.01 (19)	0.48±0.02 (20)	B D C E
November–December	0.13±0.01 (20)	0.23±0.02 (19)	0.12±0.01 (18)	0.27±0.02 (19)	D B C E

1 % increase in body length per day.
2 Letters in brackets represent the experimental groups in the range test.
3 Groups with common underscoring are not significantly different.
4 All data are $\bar{X}$±SEM.
5 Numbers in brackets are sample sizes.

Table 3.4. Results of the factorial analysis of variance showing the significant effects of temperature, photoperiod, and the temperature–photoperiod interaction on serum growth hormone (GH) levels on Day 10 and Day 30, and somatic growth rates (SGR) and linear growth rates (LGR) on Day 30. Values of *P* are indicated where a significant effect was detected; nonsignificance (NS) is based on $p>0.05$.

		Experiment					
Variable	Source	3.1	3.2	3.3	3.4	3.5	3.6
Day 10 GH	Photoperiod	NS	.001	NS	NS	NS	.03
	Temperature	.001	.001	.001	.001	.001	.001
	Interaction	NS	.04	.01	NS	NS	.001
Day 30 GH	Photoperiod	NS	NS	NS	.01	NS	.001
	Temperature	.001	.04	.001	.001	.001	.001
	Interaction	NS	NS	.01	NS	.03	.001
SGR	Photoperiod	.001	NS	.001	NS	.03	NS
	Temperature	.001	.001	.02	.02	.001	NS
	Interaction	.01	NS	.01	NS	NS	.04
LGR	Photoperiod	.001	NS	.001	NS	.02	NS
	Temperature	.001	.001	.001	.01	.001	.001
	Interaction	.001	NS	.01	NS	NS	NS

Figure 3.2. Serum growth hormone (GH) levels in goldfish maintained under different environmental regimes in March–April. All values are $\bar{X}\pm SEM$. The initial sample represents the serum GH level in fish sampled at the start of the experiment. Significant differences between Day 10 and Day 30 in serum GH levels under each experimental regime are indicated by '*' ($p<0.01$). The results of Duncan's multiple range test are indicated; groups with common underscoring are not significantly different ($p>0.05$). The bracketed letters below the abscissa represent each experimental group in the range test. The numbers above the abscissa are the sample size of each group. Note the $\log_{10}$ ordinate axis.



On Day 10, fish subjected to 16L:8D, 12 °C had a significantly lower serum GH level than all other groups sampled at this time. Serum GH levels in the other groups sampled at this time were not significantly different. In the 16L:8D, 12 °C group sacrificed on Day 30, the serum GH level was significantly elevated as compared to the same group sacrificed on Day 10. On Day 30, the serum GH level in the 16L:8D, 12 °C group was significantly lower as compared to the level in only the 8L:16D, 20 °C group .

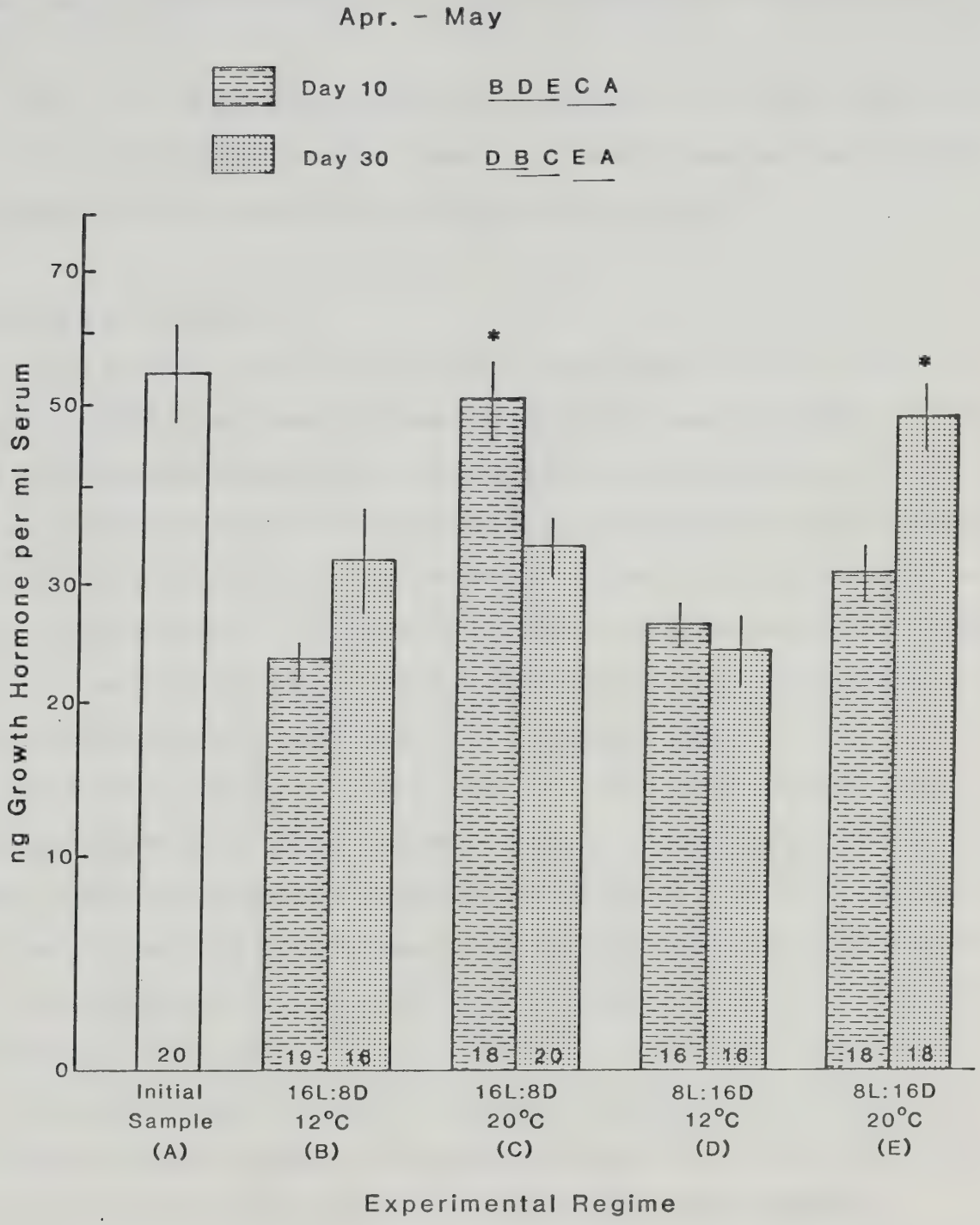
In two groups of fish exposed to 20 °C, the mean SGR values were significantly increased compared to the SGR from the groups exposed to 12 °C (Table 3.2). The highest LGR values were also found in the groups of fish exposed to 20 °C, but the increase was significant in only the 16L:8D, 20 °C group compared to the two 12 °C groups (Table 3.3). Sexual differences in growth rates were not observed in any experimental group (data not shown).

The factorial analysis of variance (Table 3.4) detected a significant interaction of temperature and photoperiod affecting serum GH levels on Day 10. On Day 30, however, only temperature significantly influenced serum GH levels. Temperature was also the main variable influencing the growth rates of fish in the experimental groups (Table 3.4).

Experiment 3.3 (April-May)

Figure 3.3 shows the serum GH levels of fish sampled in the April–May experiment (Experiment 3.3). No significant differences in serum GH levels were observed between fish sampled at 08:00 and 16:00 hr, or between male and female goldfish within any experimental group (data not shown). On Day 10, the serum GH level in the group sampled at the start of the experiment was similar to the level in the 16L:8D, 20 °C group; serum GH levels in these two groups were significantly higher compared to the levels in the other groups sampled on Day 10. On Day 30, the serum GH level in the 16L:8D, 20 °C group was significantly lower than the level observed in this group on Day 10. The 8L:16D, 20 °C group on Day 30 had a significantly elevated serum GH level compared to the same group on Day 10. The group of fish sampled at the start of the experiment and the 8L:16D, 20 °C group sampled on Day 30 had similar levels of GH, and the GH levels in both these groups were significantly higher than the serum GH levels in the all other groups sampled on Day 30.

Figure 3.3. Serum growth hormone (GH) levels in goldfish maintained under different environmental regimes in April–May. All values are $\bar{X}$±SEM. The initial sample represents the serum GH level in fish sampled at the start of the experiment. Significant differences between Day 10 and Day 30 in serum GH levels under each experimental regime are indicated by '*' ($p<0.01$). The results of Duncan's multiple range test are indicated; groups with common underscoring are not significantly different ($p>0.05$). The bracketed letters below the abscissa represent each experimental group in the range test. The numbers above the abscissa are the sample size of each group. Note the $\log_{10}$ ordinate axis.



The instantaneous relative growth rates for goldfish sacrificed on Day 30 are summarized in Table 3.2 and Table 3.3. The SGR in fish exposed to 16L:8D, 20 °C was significantly higher than the SGR in the other groups. The LGR was significantly higher in the two groups of fish exposed to the warmer temperature; also, the LGR of the 16L:8D, 20 °C group was significantly higher than the LGR measured in fish exposed to 8L:16D, 20 °C. There were no significant differences in SGR or LGR between the sexes (data not shown).

On both Day 10 and Day 30, a temperature photoperiod interaction significantly influenced serum GH levels (Table 3.4). A temperature and photoperiod interaction also had a significant effect on both the SGR and LGR in this experiment.

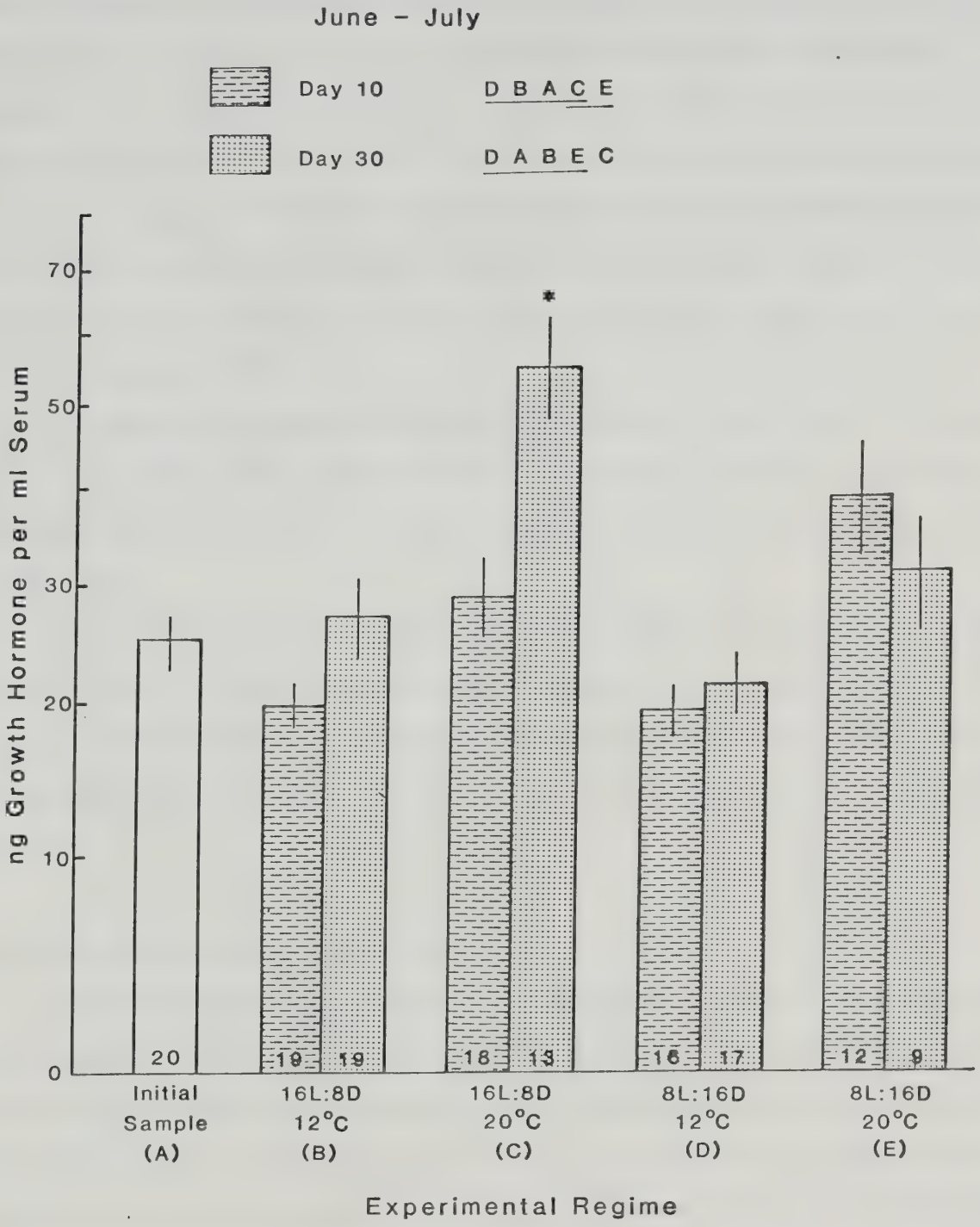
Experiment 3.4 (June-July)

Serum GH levels in fish from the June–July experiment are shown in Figure 3.4. No significant differences in serum GH levels were found between fish sampled at 08:00 and 16:00 hr, or between sexes within any experimental group (data not shown). On Day 10, the serum GH level in the 8L:16D, 20 °C group was significantly elevated compared to the serum GH levels in the group of fish sampled at the start of the experiment and the 16L:8D, 12 °C and 8L:16D, 12 °C groups. On Day 30, the serum GH level of the 16L:8D, 20 °C group was significantly higher than the serum GH level in the same experimental group sacrificed on Day 10, and all other groups sampled on Day 30.

As shown in Table 3.2, the SGR of the 8L:16D, 20 °C group was significantly increased compared to the groups exposed to 12 °C, but not compared to the 16L:8D, 20 °C group. Similar LGR values were observed in the 16L:8D, 20 °C and the 8L:16D, 20 °C groups, and the mean LGR values in these two groups were significantly higher than the LGR in the two groups at 12 °C. Significant sexual differences in the growth rates were not found (data not shown).

On Day 10, temperature was the only variable significantly influencing serum GH levels (Table 3.4). Both temperature and photoperiod significantly influenced serum GH levels on Day 30, although the interaction of these variables was not significant. Temperature was the only variable significantly influencing SGR and LGR in this experiment.

Figure 3.4. Serum growth hormone (GH) levels in goldfish maintained under different environmental regimes in June–July. All values are $\bar{X}\pm$SEM. The initial sample represents the serum GH level in fish sampled at the start of the experiment. Significant differences between Day 10 and Day 30 in serum GH levels under each experimental regime are indicated by '*' ($p<0.01$). The results of Duncan's multiple range test are indicated; groups with common underscoring are not significantly different ($p>0.05$). The bracketed letters below the abscissa represent each experimental group in the range test. The numbers above the abscissa are the sample size of each group. Note the $\log_{10}$ ordinate axis.



Experiment 3.5 (September-October)

Serum GH levels from the groups of fish sampled in this experiment are shown in Figure 3.5. There were no significant differences in serum GH levels between fish sampled at 08:00 and 16:00 hr in any experimental group (data not shown). The goldfish used in this experiment were sexually regressed (Table 3.1) and the sexes could not be visually distinguished; therefore, sexual differences in any of the variables could not be determined. On both Day 10 and Day 30, the serum GH levels in all groups were significantly lower than the serum GH level of the group of fish sampled at the start of the experiment. On Day 10, the 8L:16D, 12 °C group had a significantly lower serum GH level than fish in the 16L:8D, 20 °C and 8L:16D, 20 °C groups, whereas the serum GH level in the 16L:8D, 12 °C group was significantly lower compared to only the 16L:8D, 20 °C group. On Day 30, fish from the 8L:16D, 12 °C group had a significantly lower serum GH level than the two groups at 20 °C.

The highest instantaneous relative growth rates were found in goldfish maintained at 20 °C. The SGR and LGR of both the 16L:8D, 20 °C and 8L:16D, 20 °C groups were significantly higher compared to the growth rates of fish maintained at 12 °C (Table 3.2 and Table 3.3).

On Day 10, temperature was the main variable affecting serum GH levels, whereas on Day 30 there was a significant temperature–photoperiod interaction influencing serum GH levels (Table 3.4). Both photoperiod and temperature influenced the growth rates, although the interaction of these two variables did not significantly affect either the SGR or LGR.

Experiment 3.6 (November-December)

Serum GH levels from Experiment 3.6 in November–December are shown in Figure 3.6. Serum GH levels in male and female goldfish, and in fish sampled at 08:00 and 16:00 hr were not significantly different within any group. Serum GH levels in the group sampled initially and the 16L:8D, 12 °C sampled on Day 10 were similar, and significantly lower than in the other groups sampled on Day 10. On Day 10, the serum GH level in the 16L:8D, 20 °C group was significantly elevated compared to all other groups sampled on Day 10, and the initial sample group. On Day 30, the serum GH level in the 16L:8D, 20 ° group was

Figure 3.5. Serum growth hormone (GH) levels in goldfish maintained under different environmental regimes in September–October. All values are $\bar{X}\pm SEM$. The initial sample represents the serum GH level in fish sampled at the start of the experiment. The results of Duncan's multiple range test are indicated; groups with common underscoring are not significantly different ($p>0.05$). The bracketed letters below the abscissa represent each experimental group in the range test. The numbers above the abscissa are the sample size of each group. Note the $\log_{10}$ ordinate axis.

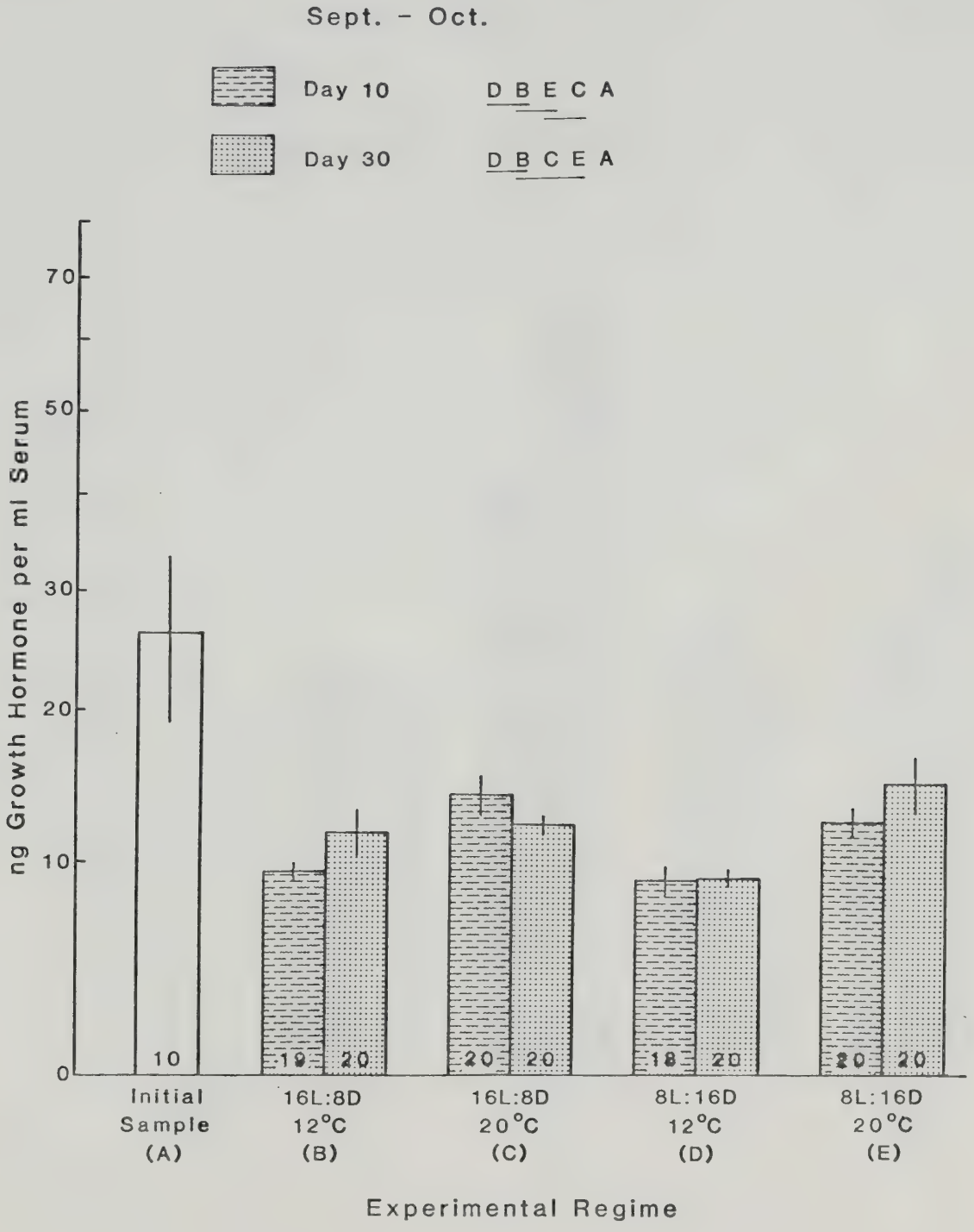
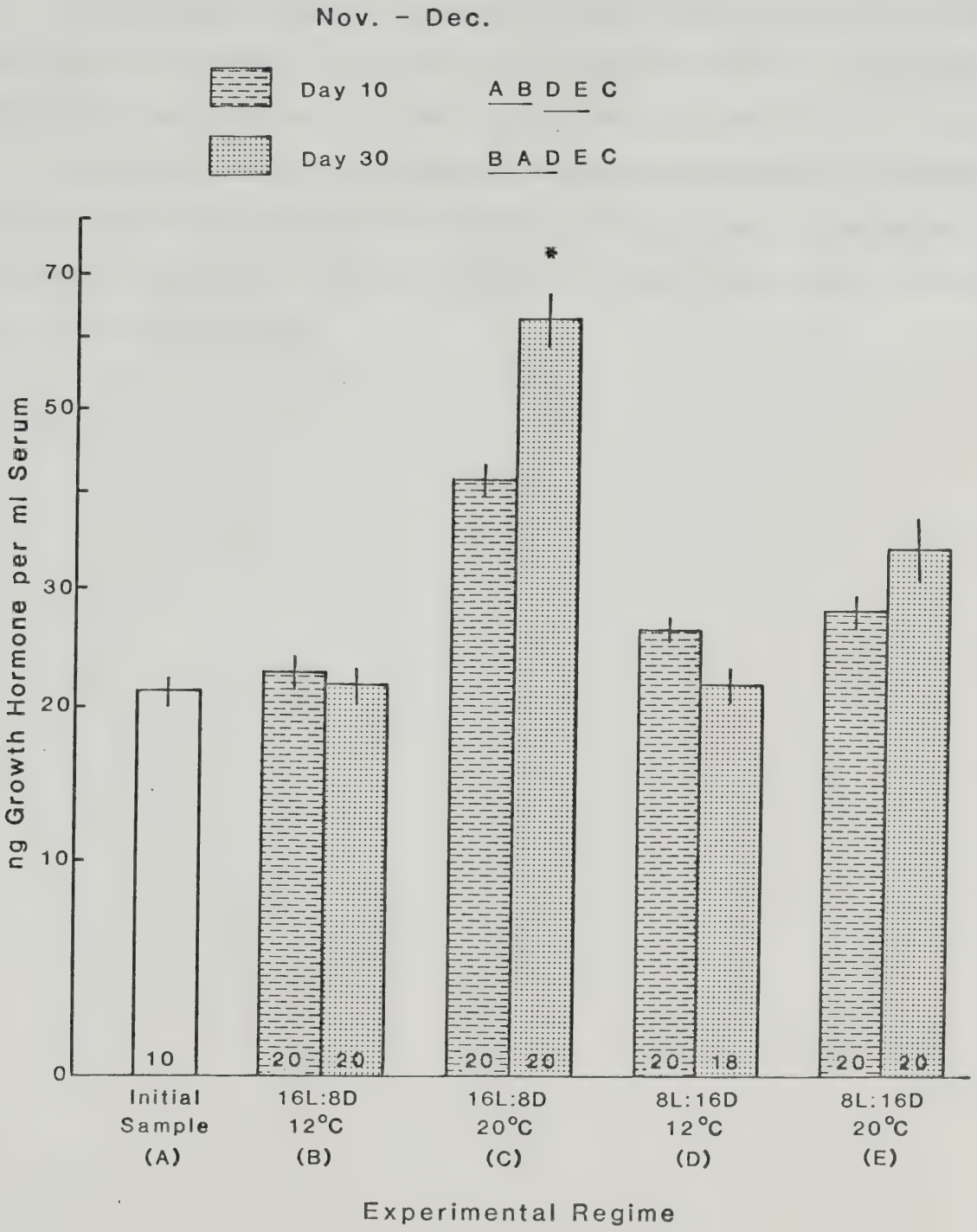


Figure 3.6. Serum growth hormone (GH) levels in goldfish maintained under different environmental regimes in November–December. All values are $\bar{X}\pm SEM$. The initial sample represents the serum GH level in fish sampled at the start of the experiment. Significant differences between Day 10 and Day 30 in serum GH levels under each experimental regime are indicated by '*' ($p<0.01$). The results of Duncan's multiple range test are indicated; groups with common underscoring are not significantly different ($p>0.05$). The bracketed letters below the abscissa represent each experimental group in the range test. The numbers above the abscissa are the sample size of each group. Note the $\log_{10}$ ordinate axis.



significantly increased compared to the serum GH levels in the same experimental group sacrificed on Day 10, the initial sample group, and all other experimental groups sampled on Day 30. The 8L:16D 20 °C group sampled on Day 30 also had a significantly higher serum GH level compared to the group sampled at the start of the experiment, and the 16L:8D, 12 °C and 8L:16D, 12 °C groups sampled on Day 30.

No significant differences in the SGR of the experimental groups were observed (Table 3.2). In contrast the LGR of the 16L:8D, 20 °C and 8L:16D, 20 °C groups were significantly higher compared to LGR values of the two groups held at 12 °C. (Table 3.3).

On both Day 10 and Day 30, there was a significant interaction of temperature and photoperiod affecting serum GH levels (Table 3.4). The temperature–photoperiod interaction also significantly influenced SGR values, whereas temperature was the main effect influencing LGR values.

D. DISCUSSION

The results of the present study indicate that temperature and photoperiod can influence serum GH levels in goldfish, and with the exception of the preliminary study by Cook (1981), the current study is the first to demonstrate the effect of these environmental parameters on circulating GH levels in a teleost species. In general, the results of the present study suggest that increased water temperature usually results in elevated serum GH levels in goldfish. However, the response to temperature was somewhat variable from experiment to experiment, and the increase in serum GH levels at the higher temperature was not always significant. The influence of photoperiod on serum GH levels was less predictable, although significant interactions of temperature and photoperiod affecting serum GH levels were evident in a number of experiments (Table 3.4).

In the present study, the experiments were conducted at various times throughout the year, and this may have influenced serum GH levels in the experimental groups. A seasonal cycle in circulating GH levels in goldfish was described previously (Chapter II), and the serum GH levels in the groups of fish sampled at the start of each experiment in the present study also demonstrated a similar pattern. The highest serum GH levels were observed in the initial groups from the March–April (Figure 3.2) and the April–May (Figure 3.3) experiments, whereas the lowest serum GH level was found in fish sampled at the start of the November– December experiment (Figure 3.6). However, the serum GH levels in the groups sampled at the start of each experiment were somewhat higher compared to the serum levels of GH in fish sampled at a comparable time of the year in the seasonal study (Chapter II). This may have resulted from the fact that fish sacrificed at the start each experiment were obtained directly from the stock aquarium, and therefore, were treated quite differently from the fish used in the experiments described in Chapter II.

Within the groups of goldfish exposed to the various environmental regimes, variations in serum GH levels from experiment to experiment also tended to follow a pattern similar to that described in the seasonal study (Chapter II). In all experimental groups, the lowest serum GH level was found in fish from the September–October experiment (Figure 3.5), approximately the same time of the year when the lowest mean daily serum GH level was found in the seasonal study (Chapter II). In addition, the highest

serum GH levels in each experimental group generally coincided with the time of year when the highest mean daily serum levels of GH were detected (Chapter II). This is especially true for the groups of goldfish maintained at 12 °C; at 20 °C, serum GH levels were more variable from experiment to experiment. These results suggest that a seasonal influence on serum GH levels was present, even after several weeks exposure to artificial temperature and photoperiod regimes. The previous enviromental history of the goldfish used in each experiment may have contributed to this observation. Prior to each experiment, the goldfish were obtained from the suppliers and, therefore, had been previously subjected to natural environmental conditions.

In most teleost species, temperature has a major influence on the rate of body growth (for review: Brett, 1979). In general, growth rates increase with increasing water temperature until an optimum temperature is reached, above which increases in temperature cause decreased growth rates (Brett, 1979). In the seasonal study (Chapter II), growth rates throughout the year in goldfish were closely correlated with changes in water temperature, with increased growth rates evident in fish maintained at the higher temperatures (Table 2.2). Temperature also had a major influence on the growth rates of the goldfish in the present study. Under the same photoperiod, goldfish subjected to 20 °C usually had increased somatic and linear growth rates as compared to fish kept at 12 °C (Table 3.2 and Table 3.3). However, photoperiod did modify the response of the growth rates to increased temperature, as evidenced by the significant effects of photoperiod, and temperature–photoperiod interaction in several of the experiments (Table 3.4). For example, in the January–February, March–April, and April–May experiments, the linear growth rate in the 16L:8D, 20 °C group was higher than in the 8L:16D, 20 °C group; the somatic growth rate was also higher in the 16L:8D, 20°C group in the January–February and April–May experiments. Later in the year, however, long photoperiod was not stimulatory to body growth; linear and somatic growth rates were similar in the 16L:8D, 20 °C and 8L:16D, 20 °C groups, or even slightly higher in the 8L:16D, 20 °C group.

In other freshwater teleost species, evidence to date also suggests that long photoperiod, or more importantly increasing photoperiod applied over several months is stimulatory to body growth, especially when applied at certain times of the year (Brett, 1979). In the present study, a seasonal effect of the stimulation of body growth by long

photoperiod is also evident in goldfish. During the early part of year when daylengths are increasing, long photoperiod is stimulatory to body growth, whereas later in the year, long photoperiods do not result in increased somatic growth.

A consistent effect of photoperiod on serum GH levels similar to that described for growth rates was not apparent in the current study. For example, during the March–April, April–May, and June–July experiments, the influence of photoperiod was highly variable (Figures 3.2, 3.3 and 3.4). Throughout the rest of the year, serum GH levels were usually similar in fish exposed to either 16L:8D or 8L:16D at the same temperature. In Chapter II, it was suggested that the relative changes in daylength throughout the year may provide the environmental stimulus by which seasonal changes in circulating GH levels are regulated. In the present study, constant photoperiod conditions were used, and it is likely that the influence of photoperiod on serum GH levels throughout the year could not be accurately assessed using the present experimental design. Future studies using photoperiods with daylengths that are increasing or decreasing with respect to the natural photoperiod cycle may provide more meaningful results regarding the influence of photoperiod on serum GH levels in goldfish. However, the photoperiod and temperature regimes used in the present study are commonly used in laboratory investigations studying hormonal changes in goldfish (Peter and Hontela, 1978; Peter *et al.*, 1978), and the serum GH levels and growth rates under the various environmental regimes determined in the present study will provide a valuable reference for future studies in goldfish using similar environmental conditions.

The growth rates in fish exposed to each set of environmental conditions were not always related to the circulating levels of GH ; increased somatic or linear growth rates were observed without a coincident increase in serum GH levels, and decreased growth rates were not always related to decreased serum levels of GH. It is possible that the lack of a relationship between circulating GH levels and growth rates in some of the experiments may be due to differences in some process related to the metabolic activity of GH, such as the metabolic turnover rate of GH or target–tissue responsiveness. In addition, exposure to the artificial environmental conditions used in the present study may have been stressful in some way to the goldfish, possibly contributing to the lack of a correlation between serum GH levels and the growth rates in some of the experiments.

In summary, increased serum GH levels were usually observed in goldfish exposed to 20 °C compared to fish maintained at 12 °C under the same photoperiod. Variations from experiment to experiment in serum GH levels in fish sampled at the start of each experiment, and in fish exposed to the same set of environmental conditions, tended to follow a seasonal pattern similar to that descibed previously in goldfish. Increased temperature usually resulted in increased growth rates, although photoperiod did modify the response of the growth rates to temperature. This was especially evident in experiments conducted in the early part of the year when fish exposed to 20 °C and a long photoperiod exhibited higher growth rates than fish exposed to 20 °C and a short photoperiod, suggesting that the effect of photoperiod on the growth rate may vary on a seasonal basis. A consistent seasonal effect of photoperiod on serum GH levels similar to that on growth rates was not observed, possibly due to the artifical nature of the constant photoperiods used in the present study.

IV. CHANGES IN CIRCULATING LEVELS OF GROWTH HORMONE DURING SPONTANEOUS OVULATION IN GOLDFISH, *Carassius auratus*

A. INTRODUCTION

Circulating levels of GH have been shown to increase around the time of ovulation in a number of tetrapod species, including humans (Genazzani *et al.*, 1975), rats (Dickerman *et al.*, 1972; Ojeda and Jameson, 1977), and turkeys (Scanes *et al.*, 1979). In addition, changes in plasma levels of GH associated with spawning in a teleost species, the white sucker, *Catostomus commersoni*, have been recently described (Stacey *et al.*, 1983a). In suckers, plasma GH concentrations are significantly elevated in ovulated and spent fish as compared to prespawning animals. The report by Chang *et al.* (1982) that a synthetic analogue of mammalian luteinizing hormone–releasing hormone, des–Gly^{10}, [D–Ala^{6}]–luteinizing hormone–releasing hormone ethylamide (LHRH–A), increased serum levels of GH in female goldfish also suggests that, in teleost fishes, GH may be influenced by reproductive activities.

Ovulation in goldfish usually occurs during the latter part of the scotophase of a 16L:8D photoperiod. A preovulatory surge of GtH begins in the first half of the photophase preceding ovulation and peak circulating levels of GtH are reached during the scotophase (Stacey *et al.*, 1979). The present report desribes changes in plasma levels of GH associated with the ovulatory surge of GtH in goldfish, and provides further evidence that GH may be related to the reproductive activities of teleost fishes.

B. MATERIALS AND METHODS

Experimental Animals

Mature female goldfish, of the common or comet varieties, were purchased from Grassyforks Fisheries Co., Inc., Martinsville, Indiana throughout the spawning season of 1983. The fish were maintained in 225 litre flow–through aquaria (12–14 °C; 16 hours light and 8 hours dark photoperiod, 16L:8D), lights on at 08:00 hr and lights off at 24:00 hr) for a minimum of three weeks prior to use. The fish were fed in excess with Ewos pellets at least twice daily.

Experimental Procedures

Ovulation was initiated using the procedures of Stacey *et al.* (1979). Groups of seven to ten fish with mature ovaries (indicated by a soft distended abdomen) were selected from the stock aquaria and transferred to 70 litre flow–through aquaria (12–14 °C; 16L:8D, lights on at 08:00 hr and lights off at 24:00 hr). All fish in each aquarium were fin–clipped for individual identification. The fish were fed in excess with Ewos pellets twice daily

Following an initial acclimation period of two to three days, the water temperature in each aquarium was increased to 20 ± °C over a period of several hours on Day 2 of the experimental period. At 10:00 hr on Day 3, floating artificial vegetation, two spermiating male goldfish, and a female induced to perform spawning behavior by an injection of prostaglandin F_2alpha (Stacey, 1976), were introduced to each aquarium. The vegetation, males and prostaglandin–injected female were removed shortly after the start of the scotophase on Day 4. All fish were checked for ovulation (indicated by the release of a stream of oocytes from the ovipore following the application of gentle pressure to the abdomen) between 06:00 and 10:00 hr on Day 4. The non–ovulatory group consists of mature fish which had not ovulated by 10:00 hr on Day 4.

Sampling Procedures

Blood samples were taken from each fish at various times starting at 24:00 hr on Day 1 to 12:00 hr on Day 12 of the experimental period. Most fish were sampled at least

twice with a minimum of six hours between samples. Prior to blood sampling, the fish were anaesthetized in MS-222. Heparinized blood samples (200 to 300 ul) were taken from the caudal vasculature using a 25 gauge needle attached to a 1 ml disposable syringe. Blood samples were kept on chipped ice for a maximum of one hour and then centrifuged at 13000g for three minutes. The plasma from each sample was collected, immediately frozen on dry ice and stored at -30 °C for several weeks until assayed for GH and GtH. Capture of the fish during the scotophase was aided by the use of a dim red flashlight. During the scotophase, all fish were anaesthetized in total darkness, although subsequent sampling was done in the light.

Hormone Measurements

Plasma levels of GH were determined using the RIA described briefly in Chapter II. Plasma GtH concentrations were measured by RIA using antisera to carp GtH, as described previously (Crim *et al.*, 1976; Hontela and Peter, 1978, 1980).

Statistical Procedures

All data for plasma levels of GH and GtH were normalized using a logarithmic transformation. Statistical comparisons between hormone levels at the various times throughout the experimental period were done using analysis of variance ($p<0.05$) followed by Duncan's multiple range test ($p<0.05$; Steel and Torrie, 1960). Differences in hormone levels between ovulatory and nonovulatory groups at each of the sample times were compared using unpaired t-test for groups with unequal variances ($p<0.05$; Snedecor and Cochran, 1980). Sample sizes in the ovulated groups at 24:00 hr on Day 1, 06:00 hr on Day 3, and 12:00 hr on Day 6 were too small to permit statistical comparison, and these groups were omitted from the statistical tests. All descriptive statistics and statistical tests were done using the SPSS package (Nie *et al.*, 1975) available through the University of Alberta computing system.

C. RESULTS

Changes in plasma GH levels during the experimental period are shown in Figure 4.1. Corresponding changes in plasma levels of GtH are shown in Figure 4.2. All ovulations occurred during the scotophase of Day 4.

Significant increases in plasma GH concentrations, in the ovulatory group as compared to the non-ovulatory group, occurred between 24:00 hr on Day 3 and 10:00 hr on Day 4. Peak plasma levels of GH in ovulatory fish appeared to have been reached during the early part of the scotphase on Day 4. By 17:00 hr on Day 4, plasma concentrations of GH had decreased to levels not significantly different from those found in non-ovulatory fish. On Days 6, 8 and 12, plasma GH levels were somewhat lower in the ovulated animals, although the differences are not statistically significant. In the non-ovulatory group, plasma GH levels remained relatively unchanged throughout the experimental period, with the exception of a relatively small but significant increase during Days 3 and 4, possibly associated with the increase in water temperature occurring on Day 2.

Variations in plama GtH concentrations (Figure 4.2) in the ovulatory group are similar to those reported by Stacey *et al.* (1979). Plasma GtH levels began to increase early in the light phase of Day 3 and continued to increase until the latter part of the scotophase on Day 4. Plasma GtH concentrations decreased rapidly following ovulation and by 22:00 hr on Day 4, had returned to levels not significantly different from those in the non-ovulatory group. In fish which did not ovulate, variations in plasma GtH levels were generally small in magnitude, although there were some significant differences.

Figure 4.1. Plasma growth hormone levels in female goldfish throughout the experimental period. All values are represented as $\bar{X}$±SEM. All fish in the ovulatory group (closed triangles) ovulated during the scotophase on Day 4, whereas fish in the non-ovulatory group (open triangles) did not ovulate during the experimental period. Each sample time is represented by a number above the abscissa for comparison by Duncan's multiple range test. The results of this test are indicated; groups with common underscoring are not significantly different ($p>0.05$). Differences between the ovulatory and non-ovulatory groups at each sample time are indicated by "*" ($p<0.05$). Sample sizes (n) at the sample times consisted of 6 to 20 fish. Note the $\log_{10}$ left ordinate axis.

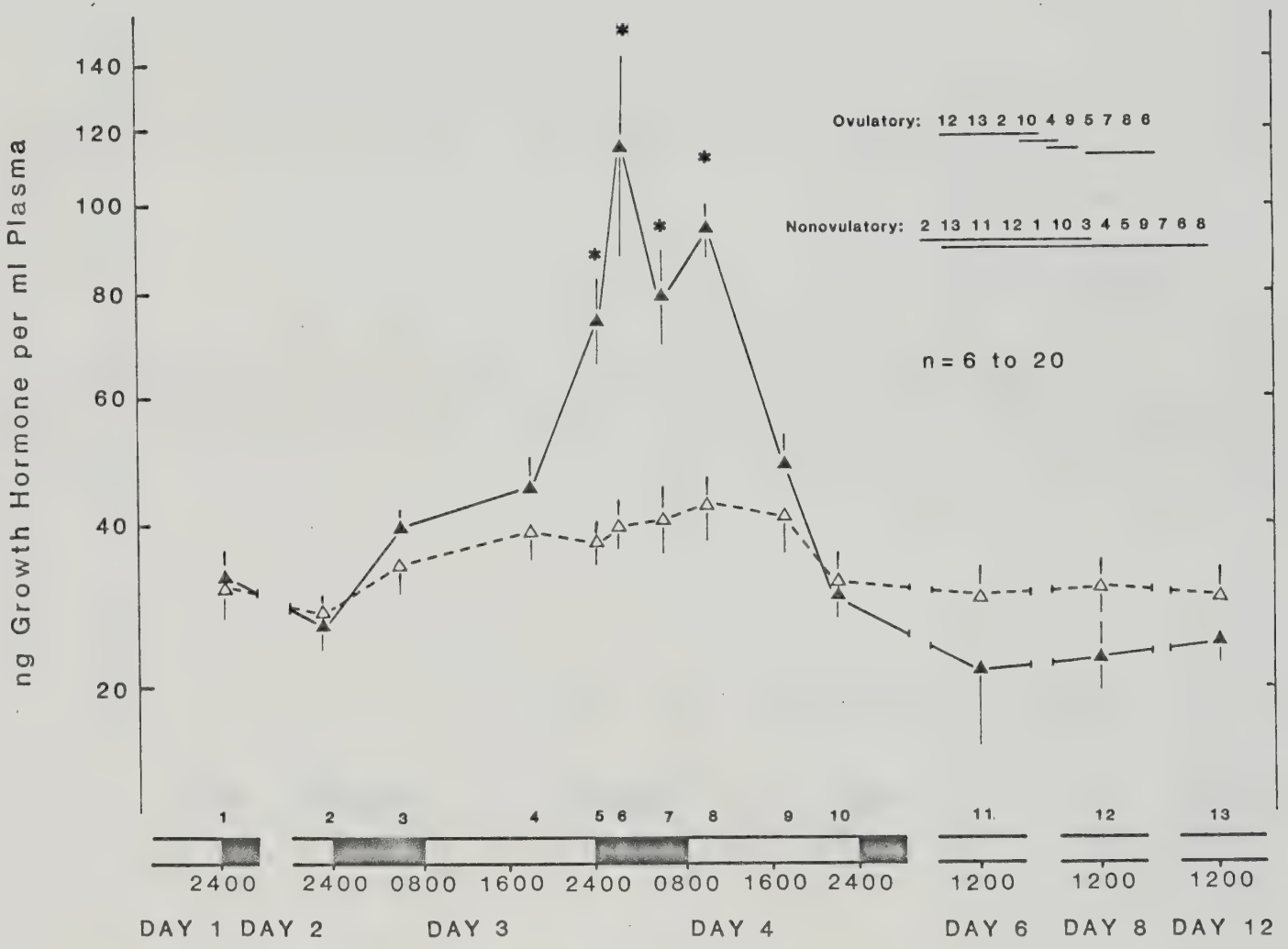
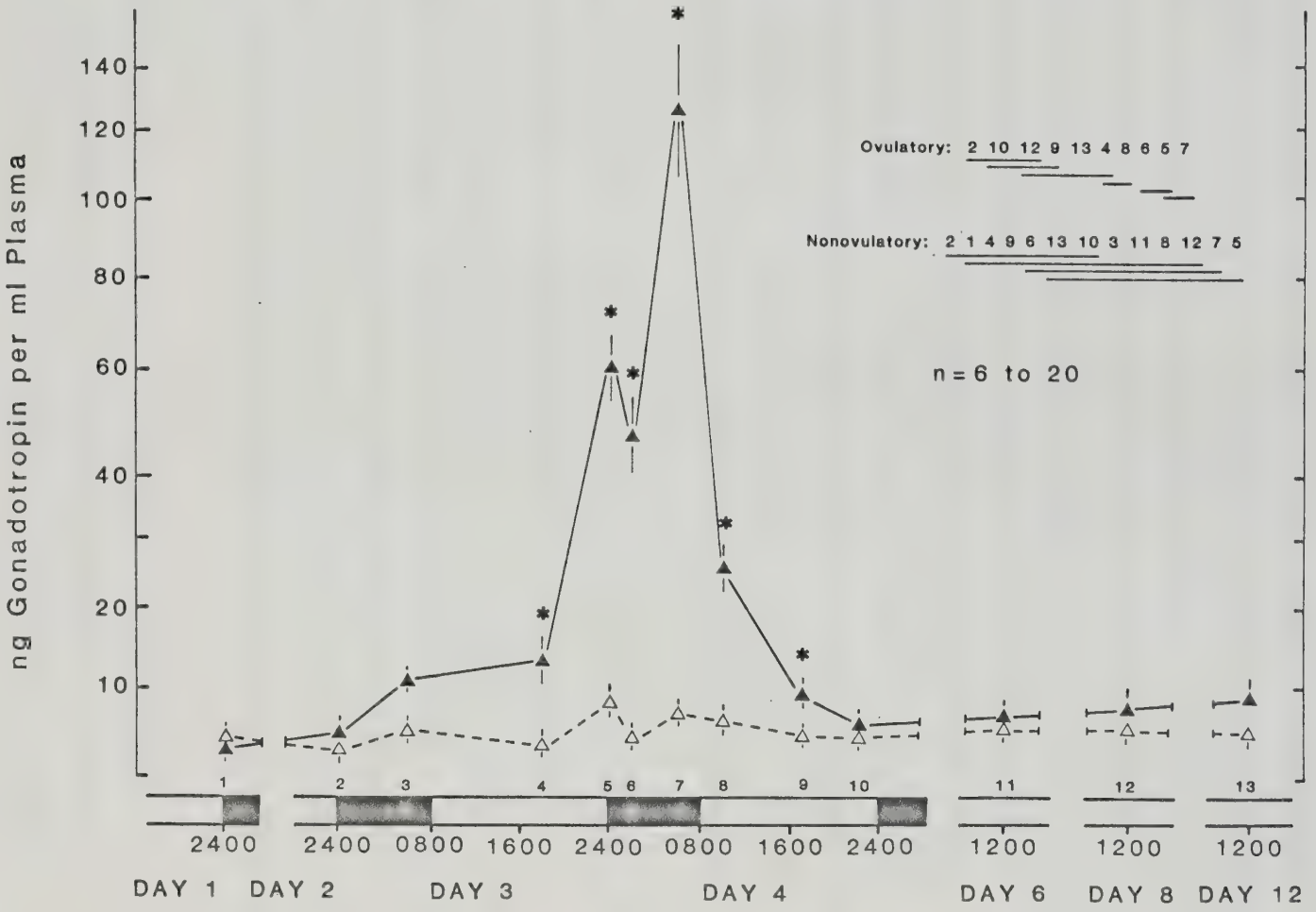


Figure 4.2. Plasma gonadotropin levels in female goldfish throughout the experimental period. All values are represented as $\bar{X}$±SEM. All fish in the ovulatory group (closed triangles) ovulated during the scotophase on Day 4, whereas fish in the non-ovulatory group (open triangles) did not ovulate during the experimental period. Each sample time is represented by a number above the abscissa for comparison by Duncan's multiple range test. The results of this test are indicated; groups with common underscoring are not significantly different ($p>0.05$). Differences between the ovulatory and non-ovulatory groups at each sample time are indicated by "*" ($p<0.05$). Sample sizes (n) at the sample times consisted of 6 to 20 fish. Note the $\log_{10}$ left ordinate axis.



D. DISCUSSION

In the female goldfish, the ovulatory surge of GtH is accompanied by a surge in plasma GH levels. Although the highest circulating levels of GH occur at approximately the same time as the peak in plasma GtH levels, there are temporal differences between the increases in the plasma levels of these two hormones. The ovulatory surge in GtH begins earlier, with increased levels of GtH occurring by about the middle of the photophase preceding ovulation; increased plasma GH levels were not detected until the onset of the scotophase. The peak in GH levels occurs early in the scotophase during which ovulation occurs; GtH levels do not peak until later in the scotophase. In addition, plasma GH concentrations remain at peak levels for at least two hours into the photophase following ovulation whereas GtH levels have decreased significantly by this time. Later in the photophase following ovulation, however, plasma GH levels have decreased to values similar to those found in nonovulatory fish. In contrast, GtH levels are still significantly elevated until about two hours before the onset of the next scotophase. Therefore, the peak in plasma GH levels may be of longer duration that the GtH peak, but the overall increase in plasma GH occurs over a shorter time period that does the ovulatory GtH surge.

The increase in plasma GH levels in ovulatory fish cannot be attributed to stresses associated with the handling of the fish or to a daily variation in circulating GH concentrations, as similar increases were not observed in non-ovulatory fish exposed to the same experimental conditions. In addition, increases in serum GH levels of the same magnitude as seen in ovulatory fish have not been detected when serum GH levels in sexually mature goldfish were measured throughout a complete 24 hour period (Chapter II). The increase in plasma concentrations of GH, therefore, must be related to the process of ovulation.

In mammals, estrogens have been shown to elevate circulating levels of GH (Dickerman *et al.*, 1972; Wiedeman *et al.*, 1976) and the periovulatory increases in circulating GH levels in tetrapods have been attributed to corresponding increases in circulating estrogens and other steroids (Dickerman *et al.*, 1972; Genazzani *et al.*, 1975; Ojeda and Jameson, 1977; Scanes *et al.*, 1979). In teleost fishes, however, the influence of estrogens and other steroids on plasma GH levels is not known. In goldfish, plasma

estradiol levels have been shown to increase during preoptic lesion-induced ovulation and spontaneous ovulation (Stacey *et al.*, 1983b). In contrast, the highest plasma levels of GH in wild spawning suckers occur in ovulated and spent animals (Stacey *et al.*, 1983a), when circulating estradiol concentrations are at the lowest levels found during the spawning period (Scott *et al.*, 1983). Further work is obviously required before the periovulatory increase in GH levels in teleost fishes can be attributed to steroidal influences.

The observation that intraperitoneal injection of LHRH-A increases serum levels of GH in female goldfish (Chang *et al.*, 1982) may be important in explaining concurrent increases in plasma levels of GH and GtH during spontaneous ovulation in goldfish. Although the actual mechanism by which LHRH-A stimulates increased serum levels of GH in goldfish is not known, it is possible that during spontaneous ovulation, endogenous hypothalamic gonadotropin-releasing hormone (GnRH) may stimulate both GtH and GH release from the pituitary. During both spontaneous ovulation (see above) and LHRH-A administration (T. Marchant and J. Chang, unpublished results), circulating levels of GtH begin to increase before GH levels and remain elevated for a longer period of time. If the somatotrophs respond directly to GnRH, the dynamics of the response is apparently different from that of the gonadotrophs. Further research is needed, however, before it is known whether or not hypothalamic GnRH is responsible for the periovulatory increase in plasma GH levels in goldfish.

In summary, the ovulatory surge of GtH in goldfish is associated with increased plasma levels of GH, although temporal differences between the peaks of GH and GtH were observed. It is suggested that hypothalamic GnRH may stimulate increased plasma levels of GH, although the mechanism of this action is not known. This study provides further evidence that GH may be related to the reproductive activities of teleost fishes.

V. GENERAL DISCUSSION

The previous chapters presented information regarding the influences of environmental variables and reproductive activities on circulating levels of GH in goldfish (*Carassius auratus*). This chapter summarizes the results from Chapter II, III and IV, and presents a general discussion of these results.

In Chapter II, variations in serum GH levels during a complete 24 hour period at various times throughout the year are described in goldfish maintained under simulated natural environmental conditions. Although daily cycles have been described for a number of hormones in goldfish (reviewed in Chapter II), evidence concerning daily changes in circulating levels of GH in teleost fishes is limited. A preliminary study in goldfish suggested that daily cycles in serum GH levels were absent, and that GH may be released in a pulsatile manner similar to that in mammals (Cook, 1981). In the current study, significant daily variations in serum GH levels in goldfish are also absent throughout most of the year. In February, however, a significant peak in circulating concentrations of GH is found shortly after the onset of the scotophase, suggesting that the peak may be determined by the start of the scotophase or the end of the photophase. At other times of the year, significant differences in serum GH levels were found between some of the sample times within the 24 hour sampling period, but the lack of duplication in serum GH levels over the next 24 hour period indicates that reproducible daily rhythms in serum GH levels are not present at these times of the year. Interestingly, the only significant daily cycle in serum GH levels was observed at the time of the year when mean daily serum GH levels are increasing, just prior to the time of the year when the serum GH levels are the highest (see below).

Previous authors (reviewed in Chapter II) have suggested that seasonal changes in circulating GH levels may be responsible for the annual cycle in body growth in teleost fishes. The present study, however, is the first to document changes in serum GH levels in relation to the annual somatic growth cycle of a teleost species (Chapter II). The highest mean daily serum GH levels were found in goldfish sampled in March and June; in July and August, mean daily serum levels of GH are decreased, with the lowest levels occurring in

fish sampled in November. These variations do not appear to be related to changes in water temperature but do correspond to seasonal changes in daylength. Therefore, it is suggested that seasonal changes in the duration of the photophase or scotophase may be the environmental factor by which seasonal changes in serum GH levels are regulated; increasing daylengths during the spring may stimulate increased serum levels of GH, whereas decreasing daylengths during the fall result in decreased serum GH levels. Future studies using photoperiods with daylengths that are increasing or decreasing with respect to the normal photoperiod may provide more evidence regarding the role of the seasonal photoperiod cycle in the regulation of circulating GH levels in goldfish.

Seasonal variations in the rates of increase in somatic weight and body length in goldfish maintained under the simulated natural environmental conditions (Chapter II) were similar to the seasonal changes in the growth rates observed in other temperate–zone teleost species (for review: Brett, 1979); growth rates in goldfish were highest during the summer and lowest in the winter months. Unlike circulating GH levels, variations in the growth rates were closely related to changes in water temperature, with the growth rates being highest in fish exposed to warmer temperatures. There was also a lag period of several weeks between seasonal maximums in serum GH levels and the growth rates. This lag period may be attributed to reduced metabolic activitiy of GH at the lower water temperatures earlier in the year; as water temperature increases, the metabolic acitvity of GH also increases. Changes throughout the year in various parameters related to the metabolic activity of GH, such as the metabolic clearance rate of GH and target–tissue responsiveness to GH, should also be studied in the future so that the relationship between seasonal changes in circulating levels of GH and the annual growth cycle in teleost fishes can be more fully understood.

The goldfish used in the seasonal study described in Chapter II were maintained at temperatures and photoperiods simulating environmental conditions (Edmonton) appropriate for the time of year during which the experiment was conducted. The laboratory studies could not exactly duplicate natural environmental conditions, and studies using a wild teleost population maintained under natural environmental conditions are needed to confirm the observations regarding changes in serum GH levels in relation to the annual growth cycle described in goldfish. However, it is interesting to note that serum GH

levels in goldfish sampled at the start of each experiment in Chapter III also followed a pattern similar to that described in goldfish sampled during the seasonal study (Chapter II), providing further evidence that a seasonal cycle in circulating GH levels is present in goldfish maintained under other environmental conditions.

The influences of constant temperature and photoperiod regimes on growth rates and serum GH levels in goldfish were studied in Chapter III. With the exception of a preliminary study in goldfish (Cook, 1981), and the circumstantial evidence presented in Chapter II regarding the role of photoperiod in the seasonal cycle in serum GH levels, the effects of these environmental factors on circulating GH levels have not been studied in a teleost species. In the experiments described in Chapter III, goldfish were exposed to artificial temperature and photoperiod regimes several times throughout the year. Increased temperatures usually resulted in elevated serum GH levels, whereas, the influence of photoperiod on serum GH levels was less predictable. In the seasonal study (Chapter II), variations in serum GH levels did not appear to be related to changes in water temperature; however, in Chapter III, increased temperature resulted in elevated serum GH levels throughout most of the year. This inconsistency may be explained by the fact that the environmental regimes used in the Chapter III experiments were artificial, and exposure to the constant photoperiod and temperature regimes may have resulted in an abnormal influence of tempertaure on serum GH levels. The influence of other water temperatures in combination with other experimental conditons (such as increasing or decreasing photoperiods) on serum GH levels should be studied in the future to clarify the role of temperature in the regulation of circulating GH levels in teleost fishes.

Increased temperature has been shown to have a stimulatory effect on body growth in teleost fishes (for review: Brett, 1979). In both the seasonal study (ChapterII) and Chapter III, temperature was also shown to have a major influence on the growth rates of goldfish, with the highest growth rates almost invariably found in fish exposed to the warmer temperatures. In Chapter III, however, photoperiod did modify the growth response to temperature, especially during the early part of the year when, at 20 °C, the highest growth rates were found in goldfish exposed to the long photoperiod. However, during the autumn and early winter long photoperiod did not stimulate higher growth rates; in fact, slighlty higher growth rates were found in goldfish exposed to a short

photoperiod, although these increases in growth rates were not significant. These results suggest that the photoperiod influence on body growth may vary on a seasonal basis, with photoperiod having a "spring" and an "autumn" effect. During the early part of the year when daylength is increasing, exposure to long photoperiod is stimulating to somatic growth (the "spring" effect). During the portion of the year when daylengths are decreasing, short photoperiod may be somewhat more stimulatory to body growth (the "autumn" effect). These results must be considered to be preliminary as the differences in the growth rates were not always significant, and a similar response to photoperiod was not observed in fish kept at the colder temperature. Research in other teleost species, however, does provide some support for the observations regarding the effect of photoperiod on the growth rates in goldfish. In general, long photoperiod, or increasing photoperiods at certain times of the year stimulate body growth in other teleost fishes (for review: Brett, 1979). Studies using other photoperiod and temperature conditions should be conducted to confirm the seasonal effect of photoperiod on body growth in goldfish described in the present study.

In the fourth chapter of this report, circulating GH levels in goldfish were found to increase during spontaneous ovulation in goldfish. The peak in plasma GH levels was very similar to the ovulatory peak in plasma GtH levels that has been observed in goldfish (Stacey *et al.*, 1979). It is not known if circulating GH has a specific role in any process related to reproduction in teleost fishes, but the results presented in Chapter IV and evidence from other studies (reviewed in Chapter IV) certainly do suggest that circulating GH levels in teleosts fishes may be related to reproductive activities. Further experiments should be conducted to determine if other aspects related to reproduction in teleosts can influence circulating GH levels. In addition, the possible influence of endogenous GnRH on GH release in teleost fishes also merits further study.

In conclusion, the present study is the first to document changes in circulating concentrations of GH in relation to the annual growth cycle of a teleost species. Although the influences of constant temperatures and photoperiods on serum GH levels were studied under artificial conditions, the role of natural changes in these environmental factors throughout the year in the regulation of the seasonal cycle in circulating GH levels remains unclear. However, the results of the present study do provide a basis for the

generation of hypotheses, which can be tested in future studies, regarding the influences of environmental conditions on circulating GH levels and growth rates in teleost fishes.

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APPENDIX I

The commercially prepared Ewos fish food (pellet size 5p) used in the present study was purchased from Astra Chemicals Ltd., Mississauga, Ontario, and the following analysis of the composition of the food was provided by the manufacturer:

Crude Protein 50.0%

Crude Fat 15.0%

Ash 10.0%

Water 9.0%

Fibre 3.0%

Carbohydrates 13.0%

B30385